simultaneously browse, add, and update FluMap information, thus providing the foundation for a powerful, community-curated knowledge base to further influenza virus research.

Construction and contents

Construction of a comprehensive, knowledge-based pathway map of influenza virus infection (FluMap)

The information used to build the FluMap (Figure 1; Additional file 1, Additional file 2, Additional file 3, and Additional file 4) was derived from several different sources. First, we manually reconstructed the Reactome 'Influenza Life Cycle' and 'Host Interactions with Influenza Virus Factors' maps [18,19] into a single map file (the FluMap pathway 'skeleton'). Next, we manually incorporated information about host pathways that are activated in response to influenza virus infection, and - for all validated interaction partners of IAV factors - we included information about downstream signalling and processing events (e.g., phosphorylation cascades). Host factor and pathway data were obtained by using published pathway maps, KEGG [20], PANTHER [23] and/or Reactome [18,19] pathway map databases. Finally, we manually integrated literature-based information regarding the influenza virus replication cycle and virus-host interactions that was absent from the Reactome pathway 'skeleton' (Approximately 13% of the interactions in the map were derived from the "skeleton", and another 10% were collected from the public pathway databases). This information was identified from review articles, extensive searches on PubMed, and text-mining platforms such as iHOP [24].

Although recent siRNA screens [2-4,6], protein-protein interaction studies [5,25-28] and global proteome analyses [29,30] have identified a substantial number of cellular factors with potential roles in the IAV infection process, FluMap includes only those with roles that have been experimentally confirmed. In addition, FluMap focuses on *intracellular* events, and does not include intercellular events (e.g., immune cell interactions). All curated reactions and interactions in the FluMap were categorized into specific parts of the influenza infection process (e.g., 'vRNP export), and for reactions imported from Reactome, we kept the reaction name from this database (e.g., 'Entry of Influenza Virion into Host Cell via Endocytosis'). A similar naming strategy was used for other reactions manually added to the map (Additional file 2 and Additional file 5).

To build the graphical representation of the FluMap (Figure 1; Additional file 2, Additional file 3, and Additional file 4), we used CellDesigner ver.4.3 [31], a modeling software that can be used to depict cellular processes step-by-step, edit annotations, and provide links to reference databases [32]; we also used Payao, a community-based, collaborative web service platform for gene-regulatory and biochemical pathway model curation [21]. The map is stored in the standard Systems Biology Markup Language (SBML) (Additional file 4), a data exchange format based on XML [33]; and it is represented in the CellDesigner's graphical notation [34], which adheres to the Systems Biology Graphical Notation (SBGN) standards [35]. Map graphics were produced using SBGN 'process description' language (Additional file 2), which allows for visualization of state transitions (e.g., stimulation or inhibition events). By using standard formats, we have enabled FluMap to be adaptable to multiple network analysis tools such as Cytoscape or to simulation by employing user-supplied kinetic laws and SBML-compliant simulators.

In addition to a detailed visual representation, we generated comprehensive, text-based annotations, which are stored in the same map file. CellDesigner enables annotation of information in three different ways: (1) in the Notes section; (2) in the MIRIAM (*M*inimum *I*nformation *R*equired *I*n the *A*nnotation of *M*odels) [36] format section; and (3) in an additional layer overlaying the base model. For FluMap, we used all three annotation options to maximise data accessibility (see Additional file 2 for details). Gene IDs, UniProt accession numbers, PubMed (reference) IDs, and Reactome IDs are stored in the Notes and MIRIAM sections. The Notes section also includes information about the intracellular location of specific interactions or reactions (e.g., 'Nucleus' or 'Mitochondria'), the stage of the infection process at which it occurs (e.g., 'Virus Entry' or 'vRNP Export'), the participation of specific viral proteins, and association with multi-protein complexes that regulate host processes (e.g., 'Apoptosome') or signalling pathways (e.g., 'MAPK'). Additional reference information (e.g., 'HA1: Yoshida R *et al.* 2009') is captured in the layer that overlays the base model. CellDesigner provides direct access to the relevant databases mentioned in the Notes section through the CellDesigner database menu, and the weblinks in the MIRIAM section by pressing the access button.

While process description diagrams capture all details of biological processes, it is also useful to have a simplified overview of the system. We, therefore, used the 'reduced notations' option in CellDesigner to illustrate the relationships between entities (positive/negative inferences, modulation, trigger, etc.). This notation depicts positive/negative influence interactions, rather than detailed events, such as phosphorylation or catalysis in the process description notation (see Additional file 2 sections B and C). Finally, we used this notation to manually construct a simplified map (Figure 2; compare to the fully detailed FluMap in Figure 1) that provides a high-level overview of the IAV replication cycle.

The FluMap is posted under http://www.influenza-x.org/flumap, where users can browse its contents using a pathway-browsing platform (iPathways+) and provide updates and improvements using a manual curation platform (Payao).

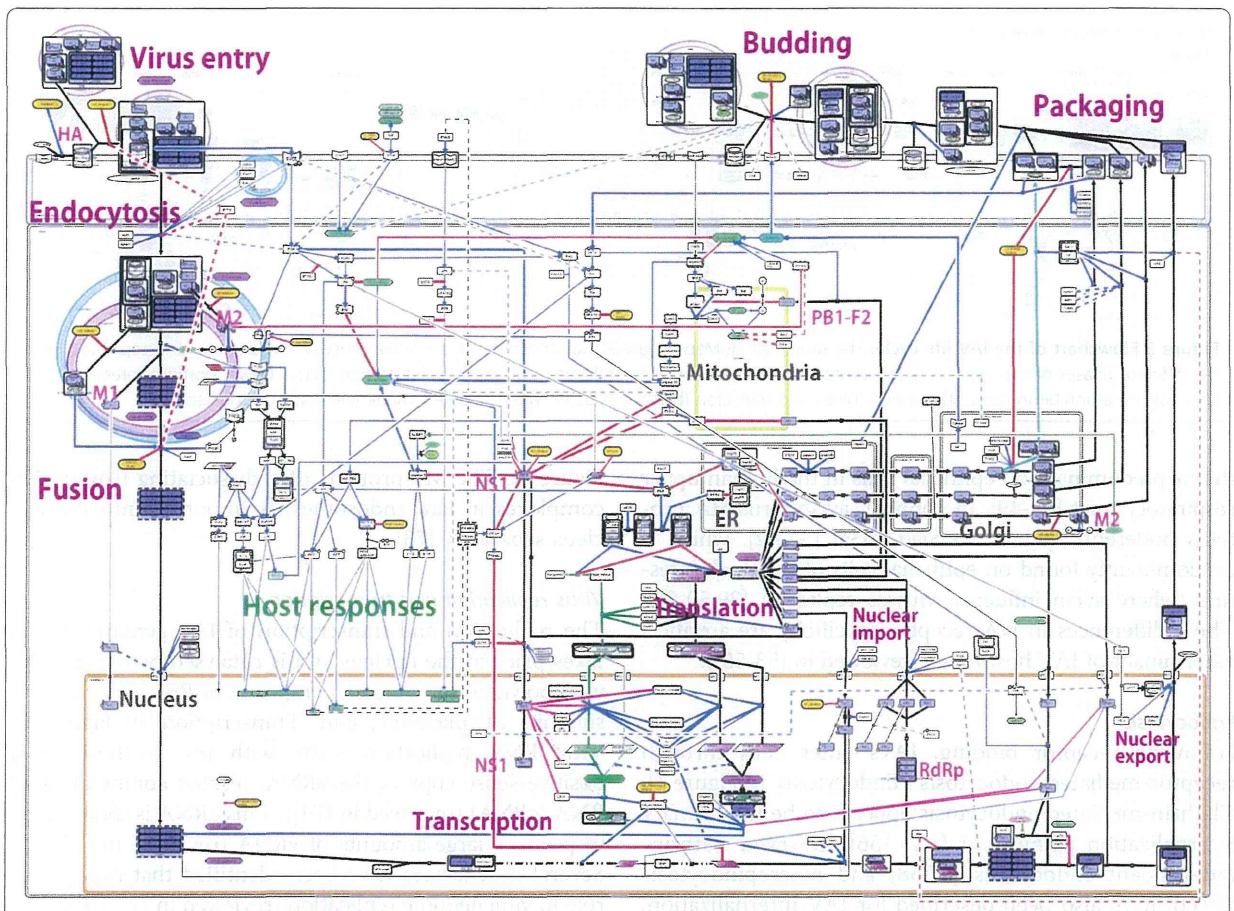

Figure 2 Simplified version of FluMap. To generate a simplified version of FluMap for a high-level overview, we extracted central components and reactions from the FluMap (virus factors (purple), host factors (green), antiviral factors (orange)), focusing on the inhibition (red) or activation (blue) of IAV replication by host factors.

General characteristics of FluMap

The comprehensive FluMap (Figure 1; see Additional file 4 for the original SBML file) contains 960 factors (696 species + 264 factors hidden in complexes) and 456 reactions. Among these, there are 558 viral and cellular proteins, 212 molecular complexes (composed of more than one component), 12 ions, 55 'phenotypes' (representing biological events such as apoptosis or autophagy), and 18 antiviral compounds. As described, all reactions are annotated with PubMed IDs in the Notes section; the entire map is annotated with 476 papers (Additional file 5 and Additional file 6). FluMap thus provides a significant improvement over the Reactome influenza infection pathway, which included 156 species and 58 reactions as of April 2012.

While the FluMap adopts the SBGN's process description graphical notation, the simplified map (Figure 2; Additional file 7) adopts the 'reduced notation' similar to SBGN's activity flow, which better facilitates visualization of the virus-host interplay at different stages of the virus life cycle. To better highlight the virus-host interplay, we manually restructured the simplified FluMap into a linear flowchart that is divided into viral and host response events (Figure 3; Additional file 8). In this representation, it is easier to track the different phases of the viral life cycle (entry, endocytosis, transcription/translation, assembly, and budding).

Description of the IAV replication cycle

In the following sections, we summarize our current knowledge of the IAV replication process as outlined in the FluMap (Figure 1), focusing on virus-host interactions.

Virus entry

The first step in the IAV life cycle is virus binding to host cells ('Virus Entry', Figure 1). The viral hemagglutinin (HA) protein is critical for this step since it binds to sialic acids on host cell glycoproteins or glycolipids. The HA proteins of human IAVs preferentially recognize sialic acid linked to galactose by an α2,6-linkage (Siaα2,6Gal) [37-42]

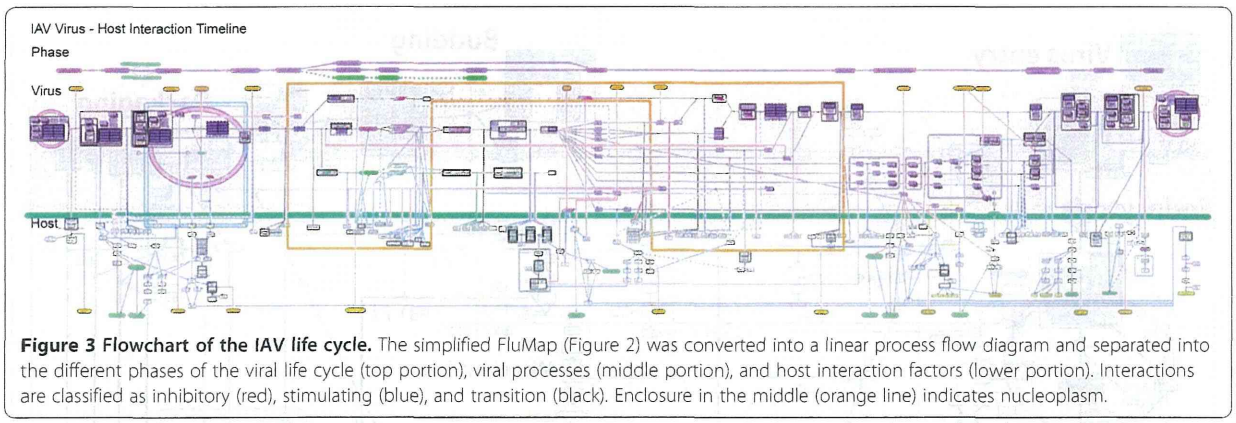

Figure 3 Flowchart of the IAV life cycle. The simplified FluMap (Figure 2) was converted into a linear process flow diagram and separated into the different phases of the viral life cycle (top portion), viral processes (middle portion), and host interaction factors (lower portion). Interactions are classified as inhibitory (red), stimulating (blue), and transition (black). Enclosure in the middle (orange line) indicates nucleoplasm.

that is predominant on epithelial cells in the human upper respiratory tract [43-49]. In contrast, avian virus HA proteins preferentially bind to Siaα2,3Gal [37-42], which is predominantly found on epithelial cells of the duck intestine (where avian influenza viruses replicate) [39,50-52]. These differences in HA receptor specificity are a critical determinant of IAV host range (reviewed in [53-55]).

Endocytosis

Following receptor binding, IAVs enter cells through receptor-mediated endocytosis ('Endocytosis' in Figure 1). Clathrin-mediated endocytosis appears to be the primary internalization pathway of IAVs [56]; however, clathrin-independent endocytosis [57,58] and macropinocytosis [59,60] have also been described for IAV internalization. Several host factors including the small GTPases Rab5 and Rab7 [61], and interferon-inducible transmembrane IFITM protein family members (i.e., IFITM1, IFITM2, IFITM3) interfere with IAV internalization [1,62].

Fusion

At the low pH of the late endosome, HA undergoes an irreversible conformational shift which expels the N-terminus of the HA2 subunit (the so-called 'fusion peptide') so that it can insert into the endosomal membrane, resulting in the fusion of the viral and endosomal membranes (reviewed in [63]) ('Fusion' in Figure 1). Through an ion channel formed by the viral M2 protein, proton influx also acidifies the interior of the virus particles, leading to the dissociation of the viral matrix protein (M1) from viral ribonucleoprotein (vRNP) complexes [64]. vRNPs are composed of one of the eight viral RNAs (vRNAs), which are wrapped around the nucleoprotein (NP) and are also associated with the viral polymerase complex (see below). Dissociation from M1 allows vRNP release into the cytoplasm and subsequent nuclear import, which is mediated by the cellular nuclear import factors importin-α (karyopherin-α) and importin-β (karyopherin-β) [65-72] ('Nuclear import' in Figure 1). The M1 protein, after dissociating from vRNP complexes in late endosomes, is imported into the nucleus separately [73].

Virus replication and transcription

The replication and transcription of IAV genomic RNAs takes place in the nucleus and is catalysed by the trimeric viral polymerase complex composed of PB2, PB1, and PA subunits ('Replication', and 'Transcription' in Figure 1). Viral RNA replication starts with the synthesis of a positive-sense copy of the vRNA, termed complementary RNA (cRNA) (reviewed in [74]). This cRNA is then copied to produce large amounts of vRNA (reviewed in [75,76]). Several host factors have been identified that may play a role in viral genome replication (reviewed in [77-79]).

Viral RNA transcription is initiated by the binding of PB2 to the 5′-cap structure of host mRNAs [80-82]. The endonuclease activity of PA [83] then 'snatches' the cap structure and the 10–13 nucleotides included with the cap serve as a primer for viral mRNA synthesis. The synthesis of viral mRNAs is carried out by the polymerase activity of PB1 [84]. The nuclear export of viral mRNAs is reviewed in York and Fodor [79]. Transcription proceeds until the polymerase complex stalls at a polyadenylation signal near the end of the viral RNA [85-88].

Two IAV mRNAs (derived from the two smallest vRNA segments, M and NS) are spliced to yield the M1 and M2, or the interferon antagonist (NS1) and nuclear export (NEP/NS2) proteins. Splicing is carried out by the host cell splicing machinery, but is likely regulated by NS1 [89,90], which binds to several cellular splicing components such as U6 small nuclear RNAs [91,92] and UAP56, a splicing factor involved in spliceosome formation [93,94].

Translation

Influenza viral mRNAs are translated by the host cell translation machinery ('Translation' in Figure 1); thus not surprisingly, several cellular translation factors such

as eIF4A (eukaryotic initiation factor-4A), eIF4E, and eIF4G interact with viral mRNAs and/or polymerase complexes [95-98]. Upon IAV infection, host cell protein synthesis is limited, and IAV mRNAs are preferentially translated [99-101]. In particular, 'cap-snatching' may deplete newly synthesized, nuclear mRNAs of their cap structures, resulting in their rapid degradation before nuclear export and translation. In addition, the interaction of NS1 with the cellular PABII (poly(A)-binding protein II) [95,98] and CPSF (cleavage and polyadenylation specificity factor) proteins [102,103], and the interaction of the viral polymerase complex with the C-terminal domain of the largest subunit of cellular DNA-dependant RNA polymerase II (Pol II) [104,105] may contribute to the inhibition of host mRNA synthesis (reviewed in [106]).

After their synthesis in the cytoplasm, the viral polymerase subunit proteins and NP are imported into the nucleus via their nuclear localization signals [71,74,107-118] to catalyse the replication and transcription of vRNA. In addition, the M1 [64,119], NEP/NS2 [120], and NS1 [121] proteins are imported into the nucleus to execute their roles in vRNP nuclear export (M1 and NEP/NS2) or the processing and export of cellular and viral mRNAs (NS1) (reviewed in [122]).

vRNP export

The nuclear export of newly synthesized vRNP complexes requires the viral NEP/NS2 [123-126] and M1 [66,127,128] proteins. The latter is thought to form a bridge between vRNPs and NEP/NS2 [129-131], and M1 association with vRNP may require M1 SUMOylation [132]. In the nucleus, vRNPs destined for export are targeted to chromatin where they associate with Rcc1, and export is mediated by the cellular export factor Crm1 ('vRNP export' in Figure 1) [125,127,133] in a manner that is likely regulated by phosphorylation [65,128,134-137]. The cellular Y box binding protein 1 (YB-1) protein also associates with vRNPs in the nucleus, is likely exported from the nucleus in conjunction with vRNPs, and facilitates vRNP association with microtubules for transport to the plasma membrane (see below) [138].

Following their synthesis by the cellular translation machinery, the viral HA, neuraminidase (NA), and M2 proteins enter the endoplasmic reticulum (ER) where they are glycosylated (HA and NA) (reviewed in [139,140]) or palmitoylated (HA and M2). Cleavage of the HA proteins of highly pathogenic avian H5 and H7 viruses (which possess multiple basic amino acids at the HA cleavage site) into the HA1 and HA2 subunits occurs most likely by cellular furin-like proteases [141] in the *trans*-Golgi network; this cleavage event is critical for the virulence of influenza viruses [142,143].

Transport of virus proteins to the cell membrane

Transport of viral proteins to the plasma membrane ('Transport to membrane' in Figure 1) likely requires MTOCs (microtubule-organizing centers) [144,145], microtubules [144-146], and additional host factors including COPI (coatomer I) protein family members [147], a Rab GTPase (Rab11A) [145,148-150], and the HIV Rev-binding protein (HRB) [151].

Packaging and budding

At the plasma membrane, HA and NA associate with lipid rafts (membrane regions rich in sphingolipids and cholesterol) that are the site of influenza virus budding [152-160] ('Packaging' and 'Budding' in Figure 1). The assembly and virion incorporation of the eight vRNPs requires segment-specific packaging signals in the viral RNAs [161,162]. The M1 protein may play a role in the assembly process since it interacts with lipid membranes [163-165], vRNPs [130,131,166] (reviewed in [167,168]), and NEP/NS2 [129,169]. In addition, some evidence suggests the possibility that the M2 cytoplasmic tail mediates vRNP incorporation into the assembling virus particle [170].

Influenza virus budding does not require the proteins of the endosomal sorting complexes that are required to transport ESCRT complexes, which are utilized by a number of other viruses for budding. Rather, M2, which is found in the raft periphery [152,157,171], appears to mediate membrane scission and particle release [172]. This process may also require the cellular F1Fo ATPase [25]. The enzymatic activity of the viral NA protein removes sialic acids from host cells and from glycoproteins on virions, allowing virus release and preventing virion aggregation (reviewed in [55,75]).

Post-translational processing

Several post-translational modifications have been described for IAV proteins, including the glycosylation of HA (reviewed in [75,142]) and NA [173], the palmitoylation (S-acylation) of HA and M2 (reviewed in [174]), and the SUMOylation (i.e., conjugation with the *s*mall *u*biquitin-like *m*odifier) of M1 [132,175], NS1 [176,177], NP [175], PB1 [175], and NEP/NS2 [175] ('Post-translational processing' in Figure 1). Moreover, phosphorylation of M1 [137,178] and NP [107,179-183] may affect vRNP nuclear import and export [66,113,128,134]. Phosphorylation of NS1 [184] and PB1-F2 (a short protein synthesized from the PB1 gene; see below) affects virulence [185], although the mechanisms are not yet fully understood. These phosphorylation events are catalysed by several cellular kinases such as PKC (protein kinase C) which phosphorylates M1 [136], PB1-F2 [185], NS1 [184,186], and PB1 [186], or by CDKs (cyclin-dependent kinases) and ERKs (extracellular signal-regulated kinases), which phosphorylate NS1 [187].

Host responses

IAV infections trigger multiple host antiviral responses (reviewed in [188,189]). These interactions are summarized in the FluMap (Figure 1) and in the flowchart that depicts the different stages of the viral life cycle (Figure 3).

As a major host defence mechanism, pattern recognition receptors (PRRs) recognize infecting agents and trigger cellular antiviral responses (reviewed in [190]). To date, three major classes of PRRs [Toll-like receptors (TLRs); RIG-I-like receptors; NOD-like receptors (NLRs)] are recognized, all of which play a role in the defence against IAV infections. The activation of PRRs leads to increased production of type I interferon (IFN) and chemokines/cytokines, resulting in the upregulation of antiviral factors.

IAV infections are recognized by TLR3 [191,192], which acts through the adaptor molecule TRIF (TIR-domain-containing adapter-interferon-beta) to stimulate IFN-regulated factor 3 and NFκB (nuclear factor-kappa beta); TLR7 [193,194], which signals through the adaptor protein MYD88 (myeloid differentiation factor 88) and induces IRF7 (interferon regulatory factor 7) and NFκB; and RIG-I [195-198], which signals through MAVS (mitochondrial antiviral signalling), also known as IPS-1, and leads to the stimulation of IRF3, IRF7, and NFκB. Moreover, IAV infection activates the inflammasome [199-203], resulting in the cleavage and activation of pro-caspase-1, interleukin-1 beta (IL-1β), and IL-18.

PRR stimulation leads to the synthesis of IFNα/β, which binds to the ubiquitously expressed IFNα/β (IFNAR) receptor, resulting in the upregulation of the JAK/STAT (janus kinase/signal transducer and activator of transcription) pathway. JAK/STAT signalling induces the formation of a transcription factor complex (composed of STAT1, STAT2, and IRF-9) that upregulates the expression of IFN-stimulated genes (ISGs). A number of ISGs encode proteins with antiviral functions, such as PKR (protein kinase R), OAS (2′-5′-oligoadenylate synthetase), RNaseL (ribonuclease L), Mx, ISG15, IFITM family members, and viperin (see below for details). IAVs have thus evolved mechanisms to counter these host anti-viral defence strategies, primarily through the actions of the NS1 and PB1-F2 proteins.

NS1 is the major viral IFN antagonist ([204]; reviewed in [189,205]). It blocks RIG-I-mediated innate immune responses by targeting RIG-I [195,206] and/or TRIM25 (tripartite motif-containing protein 25) [207], and interferes with caspase-1 activation [208].

NS1 also interferes with the effects of several antiviral host factors. IAV infection activates PKR, resulting in the phosphorylation of the eukaryotic translation initiation factor eIF2α and the subsequent shutdown of protein synthesis. This activation is inhibited by NS1 [209-214]. NS1 also controls the antiviral activity of OAS and RNaseL, a cellular nuclease that degrades viral RNA [215]. ISG15 (interferon-stimulated gene 15) is an IFNα/β-induced, ubiquitin-like protein that conjugates to a wide array of cellular proteins, thus blocking their function. It affects IAV infection by interfering with the function of NS1 [216,217].

IAV infection stimulates the phosphoinsitide-3-kinase PI3K/Akt pathway [218-226], which has pro- and antiviral functions (reviewed in [219]). In particular, this pathway is activated by NS1 binding to the p85 subunit of PI3K [218,221,224,226-228] and by IAV vRNAs via RIG-I [229]. Activation of the PI3K/Akt pathway is critical for efficient IAV replication [219,220], likely by preventing premature apoptosis [222,227,230-232].

The C-terminal four amino acids of most NS1 proteins comprise a PDZ ligand domain motif [233] that affects virulence [234-236] (reviewed in [237]), most likely through interaction with the cellular PDZ domain proteins Scribble, Dlg1 (disks large homolog 1), and membrane-associated guanylate kinase MAGI-1, -2, and -3 [238-240], which play roles in the regulation of apoptosis or tight junction formation.

NS1 also reduces the levels of IFNα/β mRNA by interfering with mRNA splicing [90-92,241] and the polyadenylation and nuclear export of cellular pre-mRNAs [90,91,102,241-246].

PB1-F2 is a short protein of 87–90 amino acids encoded by the +1 reading frame of most, but not all, IAV PB1 genes. It localizes to the mitochondrial membrane [247-249] where it interacts with the mitochondrial membrane proteins ANT3 (adenine nucleotide translocator 3) and VDAC1 (voltage-dependent anion-selective channel 1) [250], resulting in membrane depolarization [251,252] and the induction of apoptosis [247,248,250]. However, a recent study suggested that the induction of apoptosis may not be the major function of PB1-F2 [253]. Rather, PB1-F2 may interfere with the function of MAVS (mitochondrial antiviral-signalling protein) [254], and the resulting inhibition of IFN induction may contribute to PB1-F2-conferred increases in pathogenicity, inflammation, and the frequency and severity of bacterial co-infections [255-259]. In addition, PB1-F2 binding to PB1 affects the intracellular localization of the polymerase protein and reduces polymerase activity, potentially affecting virulence [260].

Other host antiviral factors include the Mx proteins [261-263], which most likely interfere with viral replication [264-266]; members of the IFITM protein family, which interfere with IAV cell entry [1,62,267]; and viperin, which executes its antiviral activity by disrupting lipid rafts that are critical for IAV budding [268].

Other important host responses to IAV infection include the mitogen-activated protein kinase (MAPK) signalling pathways, which regulate multiple cellular events

including cell cycle control, cell differentiation, and apoptosis. All four of the currently recognized MAPK pathways [extracellular signal-regulated kinases 1/2 (ERK1/2); c-jun-N-terminal kinase (JNK); p38; and ERK5] are activated upon IAV infection [135,269-276]. Some of these pathways may have both pro- and antiviral functions [135,274,277-279].

Antiviral compounds

The FluMap also captures antiviral compounds that are directed against a viral factor or a host target that is critical for efficient viral replication (reviewed in [280-283]). See Additional file 9 for a summary table.

Currently, there are two types of FDA-approved anti-IAV compounds: M2 ion channel inhibitors (amantadine, rimantadine), and NA inhibitors (oseltamivir, zanamivir).

M2 ion channel inhibitors block the ion channel in the viral envelope formed by the viral M2 protein. They prevent the influx of hydrogen ions from the acidic late endosome into the interior of the virion, a process that is necessary for the release of vRNPs into the cytoplasm. However, these inhibitors are no longer recommended for use in humans because most circulating IAVs are resistant to these compounds [284].

The NA inhibitors oseltamivir and zanamivir are the only antivirals currently recommended worldwide for human use. Both compounds block the enzymatic activity of NA that is critical for efficient virus replication [285-288]. Resistance to NA inhibitors has been described but is not widespread among currently circulating IAVs (reviewed in [289]).

Several new antiviral compounds are in different stages of clinical development and/or have been approved for human use in some countries, including two new NA inhibitors, peramivir [290,291] and laninamivir [292], and a viral polymerase inhibitor, T-705 [293-295].

Other strategies include the development of compounds that interfere with virus replication (ribavirin) [296,297], NP function (nucleozin) [298-301], NS1 function (several candidates) [302-304], or HA function [chemical compounds such as arbidol [305] that block HA-mediated membrane fusion, or monoclonal antibodies (MABs) directed against HA]. In particular, the development of monoclonal antibodies that target conserved regions of the HA protein and interfere with HA-mediated receptor-binding or fusion has received increased attention [306-314].

Host factors that are crucial for efficient IAV replication but dispensable for cell viability may be interesting drug targets since they are less likely to acquire resistance to an antiviral compound compared with IAV proteins (reviewed in [281,283]). For example, the sialidase DAS181 (Fludase, NexBio), which cleaves sialic acids on human bronchial tissue and inhibits IAV infection [315-317], is currently in Phase II clinical trials in the U.S. [283]. Several other approaches that are in early stages of development include: (*i*) protease inhibitors that block cellular enzymes required for HA cleavage [318-320]; (*ii*) specific inhibitors of MAPKs, such as U0126 (a MAPK/ERK inhibitor), which blocks the nuclear export of vRNP complexes [135,321]; (*iii*) NFκB inhibitors such as acetylsalicylic acid (ASA; commonly known as aspirin) [322], although aspirin may have adverse effects in IAV-infected individuals [323,324]; and (*iv*) agonists of sphingosine-1-phosphate (S1P) receptors, such as AAL-R, which reduce lung pathology upon IAV infection, likely because of their effect on dendritic cell activation, T-cell responses, and cytokine levels [325,326].

In silico prioritization of potential drug targets

A critical quest in infectious disease research is to identify and prioritize novel potential therapeutic targets. In our *in silico* analysis of FluMap, we exploited a specific aspect of the network called controllability to identify molecules that, when inhibited, increase the likelihood of deregulating the virus replication cycle. Controllability is the ability to drive a network from any initial state to any desired state in a finite amount of time given a suitable choice of inputs [327]. From a biological network perspective, controllability analyses identify key molecular entities and processes that when perturbed can drive a biological system from a disease state to a healthy state [328].

To begin, we identified the smallest set of driver nodes (molecules, complexes, etc.) needed to attain complete control of all of the other nodes in the network. The size of this smallest set was directly related to how difficult it was to control the network in question. Networks that demand a large set of driver nodes are inherently more difficult to control. Further, as nodes are removed from the network, the identity of the driver nodes may change but, more importantly to our application, the number of driver nodes – and the associated difficulty of controlling the network – may remain fixed or also change. Thus, the second step of the analysis involved identifying 'critical' nodes that when removed from the network, *increased* the number of driver nodes necessary to elicit complete control, that is, increase the difficulty in controlling the network [329]. From a therapeutic perspective, inhibition of critical nodes/links would make it increasingly difficult for the virus to maintain control of the replication process. Further, controllability analysis can also be performed for the network links. Lastly, we investigated whether the critical nodes/links are associated with more commonly used network topology measures (e.g., nodes with a high number of neighbours (degree) or nodes that are bottlenecks in the network (betweeness)).

To facilitate the above analyses, we converted FluMap to a binary network by taking the direction of connections while ignoring the type of reaction (catalysis, inhibition etc.) (Figure 4; Additional file 10 and Additional file 11). Note that controllability analysis does not use the type of reaction (e.g., catalysis, inhibition etc.). Thus, ignoring the type of reaction does not affect the results.

Within the FluMap, we found that 256 (41.2%) of the nodes were driver nodes and 112 (18.0%) were critical nodes. Among the 137 critical links (15.3%), ~15% accounted for interactions among viral factors, whereas ~10% accounted for virus-host interactions. The remaining two-thirds accounted for reactions between host factors. Compared with previous studies [327], the driver nodes ratio of the FluMap is similar to that of metabolic networks (30%–40%), and lower than the gene regulatory networks (>80%).

Topology analysis revealed that critical nodes tended to have a higher degree and higher betweenness than noncritical nodes (two-sided Wilcoxon rank sum test [WRST] of the degree and $\log_{10}$ of the betweenness; $P < 2.2E\text{-}16$ and $P = 3.452e\text{-}06$, respectively, see Additional file 12). By using the node degree to prioritize the critical nodes, we found that the nuclear pore complex (NPC) and the three host proteins, Akt, PKC, and the Ran/GTPase complex (which plays a critical role in the export of proteins from the nucleus to the cytoplasm), are both critical and highly connected within the network. PKR and Y-box binding protein 1 (YB-1) come in the second tier. YB-1 is reported to assist in the transport of influenza virus RNP to microtubules [138]. Perturbation of these complexes/factors would thus be expected to have the greatest impact on the IAV life cycle.

Among the *137 critical interactions* identified, we did not find that critical interactions have a higher or lower edge betweeness than noncritical interactions (P=0.1, WRST of the $\log_{10}$ of the edge betweenness), but we did find that the ISG15-NS1 interaction and several interactions related to pH control involved molecules with high degree. Our controllability analysis identified several current antiviral compounds and targets, such as M2 ion channel inhibitors (which affect the pH inside the virion), the targets of sialidase, and viral polymerase inhibitors (Figure 4).

Our results suggest that the controllability analysis, together with network topology characteristics, can identify important factors for the viral life cycle that may be potential therapeutic targets as well as known drug targets. Given that the current map is constructed by manual curation, many important edges and nodes may be missing, so that the robustness of the controllability analysis cannot be assessed. Nonetheless, we show the potential of identifying and prioritizing critical nodes and edges that may be targeted for antiviral drug development.

Utility and discussion

Here, we present FluMap, a comprehensive pathway map for IAV infections. This map is the most recent version of the IAV host-virus interaction map and includes

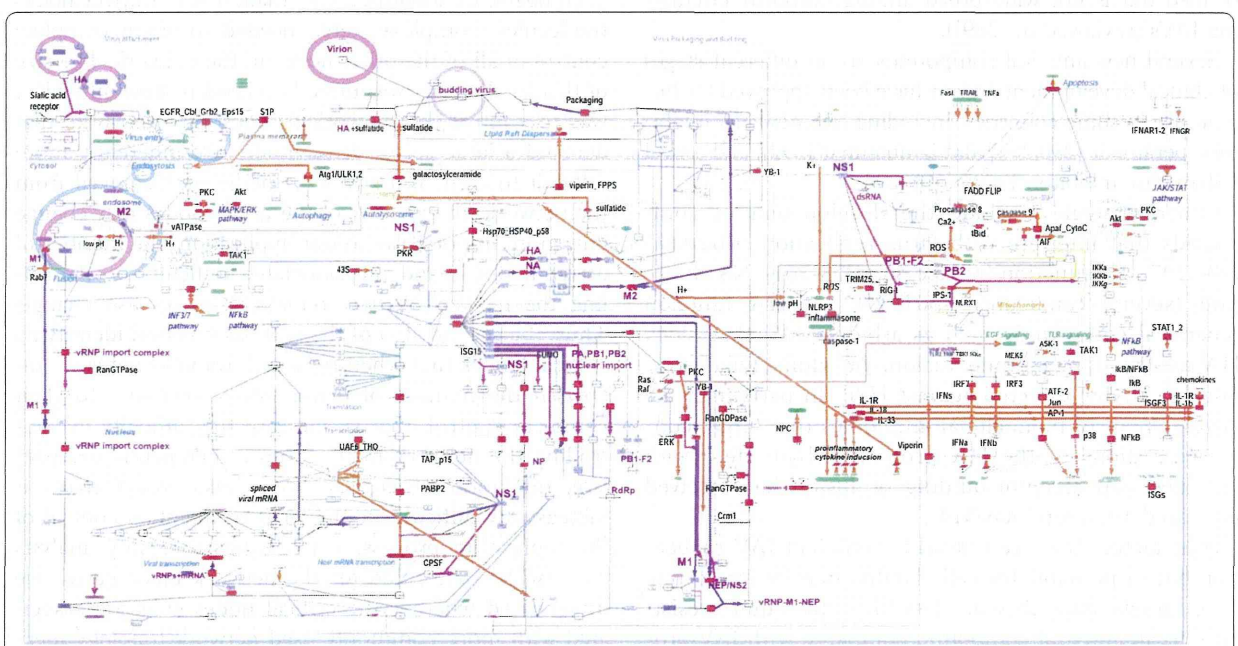

Figure 4 Controllability analysis. Critical factors (nodes) identified by the controllability analysis are shown in red. Thick magenta edges indicate critical interactions between viral and host proteins, purple edges indicate virus factor interactions/transitions (for example, transport processes), and orange edges indicate interactions between host factors.

a significantly higher number of factors than previous versions. It is intended to provide a platform for data sharing, community curation, and *in silico* analysis, such as network controllability analysis. We have made FluMap accessible online to allow for pathway and annotation browsing. We have also provided interactive features that will allow the research community to actively participate in improving and updating FluMap.

FluMap as a data analytic platform

We applied a network controllability analysis to demonstrate that maps like FluMap can be used for *in silico* analysis. Although the controllability analysis we applied here does not take into consideration the nature of the interaction (for example, activating or inhibitory), our analysis identified several events known to be critical for the IAV life cycle, suggesting that the algorithm [327] can be effectively applied to process-descriptive pathway networks such as FluMap to identify and prioritize factors that could be targeted to affect the IAV life cycle. In addition to known targets, our analysis also identified factors that are not currently recognized as critical, such as YB-1; further experimental testing could address the significance of these events in IAV infections.

A comprehensive map such as FluMap can also be used to analyze large-scale data sets (obtained from 'omics' or siRNA inhibition studies) by using the data mapping function of CellDesigner or other visualization tools.

For a deeper insight into IAV virus-host interactions, the next step in pathway modeling is the integration of additional datasets of host responses to IAV infections. FluMap includes critical host response factors such as RIG-I, PKR, and the NLRP3-inflammasome. However, the pathways regulated by these factors are complex and a significant amount of 'cross-talk' occurs between the pathways, making it extremely challenging to comprehensively map host responses. Here, the integration of additional experimental data as they become available will improve our understanding of host responses to IAV infections. Moreover, future versions of FluMap could integrate intercellular reactions, such as events stimulated by interferons and cytokines/chemokines.

Lastly, a key distinction of FluMap compared with previous influenza replication cycles is the inclusions of strain-specific information. There are strong differences between the pathogenic potential of individual virus strains, and highly pathogenic strains may exploit different host machinery to ensure rapid replication and immune suppression [330-333]. Within FluMap, users can exploit the various annotations tools to analyse isolate-specific pathway interactions and attempt to identify critical molecular events associated with highly pathogenic infections. As future studies with H5N1, H7N9, or reconstructed Spanish influenza viruses reveal more information regarding virus-host interactions, the FluMap presented here will provide a basis for rapid consolidation and *in silico* exploration.

Conclusions

We constructed a publicly available knowledge base called "FluMap" that contains 960 factors and 456 reactions. All reactions are annotated with PubMed IDs in the Notes section and isolate-specific information is available from many interactions; the entire map is annotated with 476 papers. FluMap is a comprehensive Influenza A virus replication life cycle and host response map, and is expected to be a valuable guidance map for those who study influenza infection.

Availability and requirements

The FluMap is accessible at http://www.influenza-x.org/flumap/.

Additional files

Additional file 1: FluMap building and workflow of literature-based pathway modeling. (a) FluMap was built based on information from the literature and from several pathway databases such as Reactome, KEGG, and PANTHER. The resulting map captures the viral life cycle and host responses. Extensive annotations are provided. We then manually generated a simplified map for high-level overview, and a map in which arrows outline the sequence of events during IAV infection (i.e., binding, internalization, nuclear import, etc.). We conducted controllability and network analyses over the FluMap to identify nodes essential to the replication process. Key interactions and nodes from these analyses are highlighted. (b) Summary of the literature-based pathway modeling process that converts and integrates textual information into a graphical representation. FluMap allows the community to browse, use, and comment on the information provided; this interface with the research community is shown in green.

Additional file 2: How to browse FluMap. This document explains how to browse FluMap at the website http://www.influenza-x.org/flumap/, and shows its graphical notation scheme, as well as the annotation policy we adopted for curation of the map. It also describes how to open the map file with CellDesigner for further analysis or modification, and how to curate the map on the Payao system (http://www.payaologue.org).

Additional file 3: A poster version of FluMap.

Additional file 4: SBML map file of FluMap. The SBML map file FluMap.xml can be browsed using CellDesigner. Please download CellDesigner at http://www.celldesigner.org/, install it, and open the SBML file FluMap.xml to browse FluMap by using CellDesigner. For usage of the software, see the documentation provided at the CellDesigner website: http://www.celldesigner.org/documents.html

Additional file 5: Entities & Reactions List of FluMap. This is a list of the entities (such as proteins, genes, etc.) and reactions (interactions between entities) in FluMap.

Additional file 6: Reference List of FluMap. This contains all of the references annotated in FluMap.

Additional file 7: SBML map file of the simple version of FluMap. The SBML map file of the simplified version of the IAV virus-host interaction map can be browsed by using CellDesigner. Please download CellDesigner at http://www.celldesigner.org/. For detail usage of the

software, see the documentation provided at the CellDesigner website: http://www.celldesigner.org/documents.html

Additional file 8: SBML map file of the flowchart version of FluMap. The SBML map file of the IAV virus-host interaction timeline can be browsed by using CellDesigner. Please download CellDesigner at http://www.celldesigner.org/. For detail usage of the software, see the documentation provided at the CellDesigner website: http://www.celldesigner.org/documents.html

Additional file 9: Antiviral Drug List. This is a list of the influenza-related antiviral drugs.

Additional file 10: Controllability Analysis. This document describes the protocol for the controllability analysis we conducted with FluMap.

Additional file 11: Controllability Analysis Results. This file contains the results of the controllability analysis, listing the critical, ordinary, and redundant nodes/links.

Additional file 12: Topology Analysis Results. This file contains the results of the topology analysis based on the controllability analysis results to prioritize the target candidates.

Abbreviations

IAV: Influenza A virus; SBML. Systems biology markup language; SBGN: Systems biology graphical notation.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

YM, HK, and YK conceived the idea of FluMap. YM, HM, and MK developed the map. AJE, TW, SW, SF, GN, and TL reviewed and curated the map. TH, SG, JS, and YM conducted the controllability analysis. YM, HM, and GN wrote the manuscript. All authors approved the manuscript.

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Modeling of Rifampicin-Induced CYP3A4 Activation Dynamics for the Prediction of Clinical Drug-Drug Interactions from *In Vitro* Data

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Abstract

Induction of cytochrome P450 3A4 (CYP3A4) expression is often implicated in clinically relevant drug-drug interactions (DDI), as metabolism catalyzed by this enzyme is the dominant route of elimination for many drugs. Although several DDI models have been proposed, none have comprehensively considered the effects of enzyme transcription/translation dynamics on induction-based DDI. Rifampicin is a well-known CYP3A4 inducer, and is commonly used as a positive control for evaluating the CYP3A4 induction potential of test compounds. Herein, we report the compilation of *in vitro* induction data for CYP3A4 by rifampicin in human hepatocytes, and the transcription/translation model developed for this enzyme using an extended least squares method that can account for inherent inter-individual variability. We also developed physiologically based pharmacokinetic (PBPK) models for the CYP3A4 inducer and CYP3A4 substrates. Finally, we demonstrated that rifampicin-induced DDI can be predicted with reasonable accuracy, and that a static model can be used to simulate DDI once the blood concentration of the inducer reaches a steady state following repeated dosing. This dynamic PBPK-based DDI model was implemented on a new multi-hierarchical physiology simulation platform named PhysioDesigner.

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Introduction

Cytochrome P450 enzymes (CYPs) are implicated in many clinically relevant drug-drug interactions (DDI), as the metabolism reactions catalyzed by this enzyme family are the dominant route of elimination for the majority of drugs. Inhibition of the CYPs can lead to an unwanted elevation in the blood level of drugs administered concomitantly, which can result in life-threatening adverse drug reactions [1,2]. Induction of CYP expression is not normally considered to be a safety concern, but can lead to inadequate drug efficacy [3]. For example, co-administration of rifampicin and cyclosporine results in excess metabolism of cyclosporine leading to allograft rejection in transplanted patients [4–6]. Thus, predictions of *in vivo* DDIs from *in vitro* metabolism data are becoming increasingly important during the process of preclinical drug development.

Various mathematical models have been proposed to predict potential clinical drug-drug interactions from *in vitro* data [7–10]. However, induction studies are generally more difficult to conduct compared with inhibition studies, as they need a cell-based system that allows evaluation of gene transcription and protein expression. The simplest model is one in which a static score of degree of induction is calculated from the average plasma concentration of an inducer using *in vitro* EC_{50} and E_{max} estimates [11,12]. The potential for induction-based DDI for any particular drug combination is then predicted based on the proportion of the drugs' total body clearance attributable to the enzymes induced. Dynamic models consider fluctuations in the levels of enzyme activity [13–15]. The clearance rate of substrate drugs can be dynamically altered by the acceleration of enzyme synthesis in an inducer concentration-dependent manner. A recent study indicated that a dynamic model, although not a marked improvement over the static model, tended to give better predictions for the 50 clinical DDI cases studied [16].

To date, the dynamic models reported are all indirect pharmacokinetic/pharmacodynamic (PK/PD) models [13–15] which assume that an inducer accelerates the enzyme synthesis in a concentration-dependent manner. Since enzyme synthesis is assumed to obey zero-order kinetics, the action of the inducer on enzyme synthesis starts immediately. Therefore, the gradual increase in CYP activity over several days' exposure to the inducer is attributed simply to the slow degradation rate of these

enzymes. However, several studies have indicated that it takes at least a few days for the mRNA to reach a maximum level [17,18]. To evaluate the kinetics of enzyme induction, it is important to consider the time courses of sequential mRNA and enzyme synthesis.

The present study is aimed at developing a hybrid simulation model for predicting the dynamics of induction-based DDI, in which a whole-body physiologically based pharmacokinetic (PBPK) model and an enzyme transcription/translation dynamics model are implemented. Feasibility of this hybrid model was investigated using rifampicin, a well-characterized and potent inducer of CYP3A4. Rifampicin is frequently used as a positive control or calibrator for evaluating the CYP3A4 induction potential of test compounds. Therefore, a large amount of *in vitro* rifampicin data is available in the literature. In general, cultures of primary human hepatocytes are believed to be the best *in vitro* model for simulating *in vivo* conditions. However, considerable functional variability of donor hepatocytes has been observed [19,20]. To obtain non-biased parameters regarding transcription and translation of CYP3A4, we systematically collected *in vitro* data and analyzed them using an extended least squares method [21,22] that allows the estimation of kinetic parameters while taking inter- and intra-individual variability into account. Using the parameters estimated from *in vitro* human hepatocytes, we then predicted clinical pharmacokinetics of CYP3A4 substrate drugs in the presence of concomitantly administered rifampicin.

Materials and Methods

Data Collection

The fm_{CYP3A4} values, i.e., the apparent contribution of CYP3A4 to drug oral clearance, were obtained for 15 CYP3A4 substrate drugs in a previous report [23,24]. These values were estimated from the increase in AUC_{oral} of the drugs tested resulting from the action of CYP3A4 inhibitors, as observed in 53 separate clinical DDI studies [23]. AUC_{oral} is the area under the blood concentration-time profile following oral administration. Information on the pharmacokinetics of CYP3A4 substrate drugs when co-administered with rifampicin (see Table S1) was also obtained from the literature [25–40]. The dosage regimen of oral rifampicin was also considered in the present analysis. Clinical pharmacokinetic data of rifampicin with different oral dosage regimens were obtained from a report by Acocella et al. [41]. In addition, *in vitro* rifampicin induction data of CYP3A4 mRNA expression and/or enzyme activity in primary cultures of human hepatocytes were also collected [17,42–46].

Modeling of the induction dynamics of CYP3A4 expression in human hepatocytes

Following the onset of treatment of hepatocytes with rifampicin, expression of CYP3A4 mRNA was up-regulated after an initial time delay, and reached maximum level on day 2 [17]. Taking into account that rifampicin induces expression of CYP3A4 via activation of the pregnane X receptor (PXR), a dynamic model with a putative receptor was defined using the following equations:

$$\frac{dPXR_{act}}{dt} = \frac{1+CYP_0/K_i}{1+CYP/K_i}\cdot\frac{RIF}{EC_{50}+RIF} - k_{inact}\cdot PXR_{act} \quad (1)$$

$$\frac{dRNA}{dt} = k_{rna,syn} + k_{rna,pxr}\cdot PXR_{act} - k_{\mathrm{rna,deg}}\cdot RNA \quad (2)$$

$$\frac{dCYP}{dt} = k_{cyp,syn}\cdot RNA - k_{\mathrm{cyp,deg}}\cdot CYP \quad (3)$$

where RIF, PXR_{act}, RNA, and CYP are the concentration of rifampicin, normalized amount of activated PXR, CYP3A4 mRNA level, and CYP3A4 enzyme level, respectively, CYP_0, EC_{50}, K_i, and k_{inact} are the baseline level of CYP3A4 enzyme, concentration of rifampicin at half-maximum PXR activation, the constant for negative feedback inhibition, and the inactivation rate constant for activated PXR, respectively. $k_{rna,syn}$ and $k_{rna,pxr}$ are rate constants for baseline and PXR-mediated synthesis of CYP3A4 mRNA, and $k_{rna,deg}$ is the rate constant for its sequestration, while $k_{cyp,syn}$ and $k_{cyp,deg}$ are rate constants for the synthesis and sequestration of CYP3A4 enzyme. In the model, a delay in the early phase of CYP3A4 mRNA expression after addition of the inducer was assumed to be attributable to the time required for activation of PXR, while the accelerated decay of this mRNA was thought to result from the subsequent negative feedback inhibition by PXR according to CYP3A4 level. In general, induction of mRNA and subsequent CYP3A4 enzyme levels is evaluated as the fold increase over the value observed on day 0. If the levels of mRNA and enzyme return to their original values (RNA_0 and CYP_0, respectively) by the removal of a stimulus, the following relationships should be satisfied:

$$k_{cyp,syn}\cdot RNA_0 = k_{\mathrm{cyp,deg}}\cdot CYP_0 \quad (4)$$

$$k_{rna,syn} = k_{\mathrm{rna,deg}}\cdot RNA_0 \quad (5)$$

Therefore, using $RNA' = RNA/\mathrm{RNA}_0$ and $CYP' = CYP/CYP_0$, Eqs. 1–3 can be replaced with:

$$\frac{dPXR_{act}}{dt} = \frac{1+p}{1+p\cdot CYP'}\cdot\frac{RIF}{EC_{50}+RIF} - k_{inact}\cdot PXR_{act} \quad (6)$$

$$\frac{dRNA'}{dt} = k_{\mathrm{rna,deg}}\cdot(1+q\cdot PXR_{act} - RNA') \quad (7)$$

$$\frac{dCYP'}{dt} = k_{cyp,\mathrm{deg}}\cdot(RNA' - CYP') \quad (8)$$

where

$$p = CYP_0/Ki \quad (9)$$

$$q = \frac{k_{rna,pxr}\cdot k_{\mathrm{cyp,syn}}}{k_{rna,\mathrm{deg}}\cdot k_{\mathrm{cyp,deg}}\cdot CYP_0} \quad (10)$$

Since the considerable inter-donor variability of drug metabolism by human hepatocytes has been attributed to variations in the baseline level of the CYP3A4 activity present [20], an extended least square analysis was performed by considering the effect of inter-donor variability on CYP_0 (that is, p and q). This analysis was carried out using the ADVAN9 routine in NONMEM 7.2 (Icon, Inc., Dublin, Ireland).

Conventional modeling of the induction dynamics of CYP3A4 activity

An indirect effect model for enzyme induction [13–15] can be represented as follows:

$$\frac{dCYP}{dt} = k_{syn} \cdot \left(1 + \frac{E_{\max} \cdot RIF}{EC_{50} + RIF}\right) - k_{\deg} \cdot CYP \qquad (11)$$

where k_{syn} and k_{deg} are rate constants for baseline synthesis and sequestration of CYP3A4 enzyme, respectively. Assuming that the level of enzyme prior to administration of rifampicin (CYP_0) was under the steady state, the following relationship should be satisfied:

$$k_{syn} = k_{\deg} \cdot CYP_0 \qquad (12)$$

Replacing the k_{syn} of Eq. 11 and normalizing it with CYP_0 ($CYP' = CYP/CYP_0$), we obtain:

$$\frac{dCYP'}{dt} = k_{\deg} \cdot \left(1 + \frac{E_{\max} \cdot RIF}{EC_{50} + RIF} - CYP'\right) \qquad (13)$$

An extended least square analysis was performed by considering the effect of inter-donor variability on E_{max}. This analysis was carried out using the ADVAN9 routine in NONMEM 7.2.

Analysis of CYP3A4 activity induction by a simple static model

Using only 72-h data, the E_{max} and EC_{50} values for induction of CYP3A4 by rifampicin were estimated from the following equation:

$$CYP' = 1 + \frac{E_{\max} \cdot RIF}{EC_{50} + RIF} \qquad (14)$$

An extended least square analysis was performed by considering the effect of inter-donor variability on E_{max}, similarly to the case of the indirect effect model mentioned before.

Modeling of the clinical pharmacokinetics of rifampicin following repeated oral dosing

PBPK models are mechanistically rigorous models that incorporate anatomical and biochemical information into descriptions of pharmacokinetics. To construct PBPK models, measurements of drug concentrations in each organ and tissue are required. However, only blood and urine data are generally available in clinic. As an intermediate approach, a PBPK model which gives an abstracted blood compartment and considers only recirculation between blood and liver has been utilized [7]. It has been demonstrated that the simplified PBPK model allows *in vitro-in vivo* extrapolation of hepatic drug metabolism [7]. Rifampicin clearance is known to be a nonlinear saturable process that accelerates during repeated oral dosing [41]. The simplified PBPK model was modified taking this specialized aspect of rifampicin pharmacokinetics into consideration. The mass-balance equations were:

$$V_1 \frac{dC_b}{dt} = -Q_h \cdot C_b + Q_h \cdot \frac{C_h \cdot R_b}{K_{p,h}} - CL_r \cdot C_b \qquad (15)$$

$$V_h \cdot \frac{dC_h}{dt} = Q_h \cdot C_b - Q_h \cdot \frac{C_h \cdot R_b}{K_{p,h}} - \frac{V_{\max} \cdot fu_p \cdot C_h / K_{p,h}}{K_m + fu_p \cdot C_h / K_{p,h}} + \sum^{t_i < t} k_a \cdot F_a F_g \cdot D \cdot \delta(t_i) \cdot \exp(-k_a \cdot (t - t_i)) \qquad (16)$$

$$\frac{dV_{\max}}{dt} = k_{in} \cdot (1 + F \cdot fu_p \cdot C_h / K_{p,h}) - k_{out} \cdot V_{\max} \qquad (17)$$

where C_b and C_h are concentrations of the drug in the blood and liver, respectively, and V_{max} is the inducible maximum hepatic elimination rate. V_1, CL_r, Q_h, R_b, and $K_{p,h}$ are the volume of systemic circulation, renal clearance, hepatic plasma flow rate, blood/plasma distribution ratio, and liver/plasma distribution ratio, respectively. V_h, fu_p, K_m, k_a, F_aF_g, and D are the volume of liver, fraction unbound in plasma, Michaelis-Menten constant for hepatic elimination, absorption rate constant, product of fraction absorbed and intestinal availability, and amount of oral dose, respectively. k_{in}, k_{out}, and F are the rate constant for synthesis of hepatic elimination activity, rate constant for decay of hepatic elimination activity, and coefficient for auto-induction, respectively. CL_r and fu_p were assumed to be 1.8 L/h and 0.2 [47], respectively. Q_h and V_h were assumed to be 96.6 L/h and 1.4 L, respectively [7]. Assuming that F_aF_g and R_b were both at unity, V_1, K_m, k_a, $K_{p,h}$, k_{in}, k_{out}, and F were estimated by curve-fitting to blood concentration-time profiles following repeated oral dosing of rifampicin with different doses [41]. The parameter estimation procedure was carried out with the ADVAN9 routine in NONMEM 7.2.

Simulation of drug-drug interactions with rifampicin

In the case of drugs that are mostly metabolized by the liver, induction-based DDI occurring after oral administration is represented by:

$$\frac{AUC^{ind}}{AUC} = \frac{1}{fm_{CYP3A4} \cdot IR + (1 - fm_{CYP3A4})} \qquad (18)$$

where AUC and AUC^{ind} are areas under the blood concentration profile in the absence and presence of an inducer, respectively. fm_{CYP3A4} and IR are the fraction of the drug metabolized by CYP3A4 and the relative activity of CYP3A4 induced by the inducer, respectively. This equation has been derived using the following assumptions: the substrate drug is eliminated solely by the liver, and the induction of CYP3A4 in the intestine is negligible. The fm_{CYP3A4} values for each substrate drug were obtained from the literature [23,24]. In the previous article [24], 53 induction-based DDI data sets in human were collected and compiled without any normalization, demonstrating that the degree of DDIs could be comprehensively explained by the IR values of various inducers determined from *in vivo* data by taking simvastatin as a standard CYP3A4 substrate. In the present study, only data for rifampicin were taken from the compiled data. The IR value for rifampicin was estimated using *in vitro* parameters with the following process: Using Eqs. 15–17, the unbound concentration of rifampicin in the liver ($fu_p \cdot C_h / K_{p,h}$) was computed. By substituting it for the variable RIF in Eq. 6 or 13, a time-course for the degree of induction of CYP3A4 (CYP') *in vivo* was estimated by Eqs. 6–8 or Eq. 13. The IR was defined as the average of CYP' over the interval.

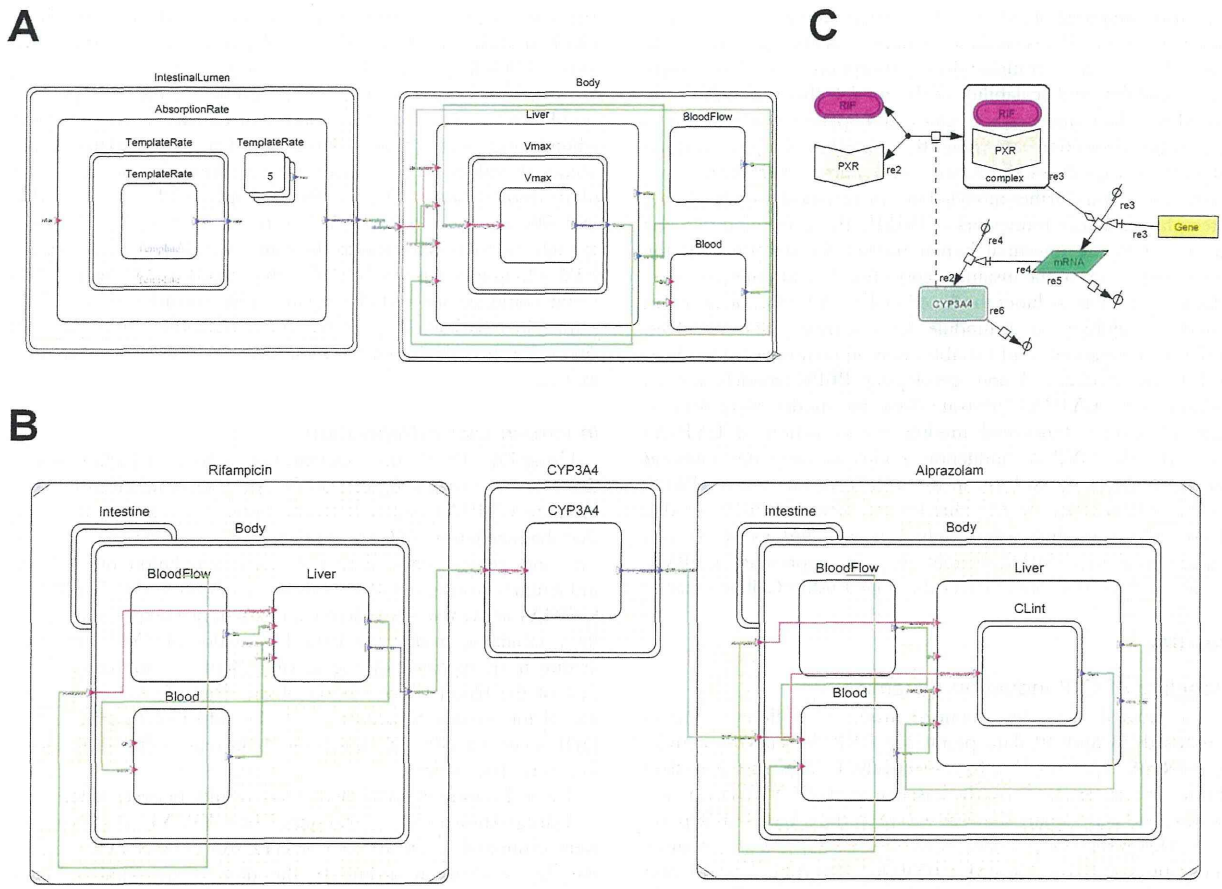

Figure 1. Snapshots of DDI models implemented in multi-hierarchical physiology simulation platforms. Fig. 1A represents a PBPK model for rifampicin implemented on PhysioDesigner, and Fig. 1B represents a transcription/translation dynamics model for CYP3A4 following administration of the drug, as implemented on CellDesigner. Fig. 1C represents a PBPK-based DDI model, where the enzyme induction model was hybridized. Yellow and white rectangles represent the capsule module and functional module, respectively. Modules can communicate by connecting their ports with an edge.
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To simulate the blood concentration-time profile of a CYP3A4 substrate drug in the presence of rifampicin, a PBPK model for the substrate, similar to that for rifampicin (Eqs. 15–17), was considered. Assuming that hepatic elimination is linear, the mass-balance equation for the liver was replaced with:

$$V_h \cdot \frac{dC_h}{dt} = Q_h \cdot C_b - Q_h \cdot \frac{C_h \cdot R_b}{K_{p,h}} - CL_{\mathrm{int},h} \cdot fu_p \cdot C_h / K_{p,h} + \sum^{t_i < t} k_a \cdot F_a F_g \cdot D \cdot \delta(t_i) \cdot \exp(-k_a \cdot (t - t_i)) \quad (19)$$

where $CL_{int,h}$ depicts intrinsic clearance for the substrate. Pharmacokinetic parameters for CYP3A4 substrates were obtained by curve-fitting to their blood concentration profiles as has been reported previously [7]. For simulation of DDI with rifampicin, $CL_{int,h}$ was assumed to be dependent on CYP':

$$CL^{ind}_{\mathrm{int},h} = CL^{0}_{\mathrm{int},h} \cdot (fm_{CYP3A4} \cdot CYP' + (1 - fm_{CYP3A4})) \quad (20)$$

where $CL^{ind}_{\mathrm{int},h}$ and $CL^{0}_{\mathrm{int},h}$ are intrinsic clearances for the substrate in the presence and absence of rifampicin, respectively. Thus, two PBPK models for the inducer and substrate were bridged with the CYP3A4 induction dynamics model to compute the DDI with rifampicin.

PHML-SBML hybrid simulation

Physiological Hierarchy Markup Language (PHML) is a markup language that can explicitly describe the multi-level hierarchical structures of physiological functions in mathematical models. One of the remarkable features of PHML is that it enables the embedding of Systems Biology Markup Language (SBML) [48] models as a module. To make a DDI model more readable and reusable, two PBPK models for both inducer and substrate were stored in the PHML format, and connected to each other via a functional module representing subcellular enzyme induction, of which the contents were implemented in SBML. The PHML and SBML models were developed using open source modeling platforms, PhysioDesigner (formerly *insilico*IDE) and CellDesigner, respectively [49,50]. PhysioDesigner and CellDesigner are freely available at http://physiodesigner.org and http://celldesigner.org.

Fig. 1 represents snapshots of the DDI model implemented in the simulation platform. As shown in Fig. 1A, a PBPK model is