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Human and chicken TLR pathways: manual curation and computer-based orthology analysis

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Abstract

The innate immune responses mediated by Toll-like receptors (TLR) provide an evolutionarily well-conserved first line of defense against microbial pathogens. In the Reactome Knowledgebase we previously integrated annotations of human TLR molecular functions with those of over 4000 other human proteins involved in processes such as adaptive immunity, DNA replication, signaling, and intermediary metabolism, and have linked these annotations to external resources, including PubMed, UniProt, EntrezGene, Ensembl, and the Gene Ontology to generate a resource suitable for data mining, pathway analysis, and other systems biology approaches. We have now used a combination of manual expert curation and computer-based orthology analysis to generate a set of annotations for TLR molecular function in the chicken (*Gallus gallus*). Mammalian and avian lineages diverged approximately 300 million years ago, and the avian TLR repertoire consists of both orthologs and distinct new genes. The work described here centers on the molecular biology of TLR3, the host receptor that mediates responses to viral and other double-stranded polynucleotides, as a paradigm for our approach to integrated manual and computationally based annotation and data analysis. It tests the quality of computationally generated annotations projected from human onto other species and supports a systems biology approach to analysis of virus-activated signaling pathways and identification of clinically useful antiviral measures.

Introduction

Proteins of the Toll-like receptor (TLR) family mediate the initial interactions of microbial pathogens—viruses, bacteria, fungi, and protozoa—with cells of an infected host, initiating signaling processes central to the host's innate and adaptive immune responses (e.g., Kawai and Akira 2005; Iwasaki and Medzhitov 2004, 2010). TLR function plays a central role in both host defense against infection and pathological processes such as host toxic reactions to bacterial lipopolysaccharide. TLR signaling processes have presented an attractive target for the development of therapeutics (Ulevitch 2004). Recent modeling studies aimed at identifying points in the overall process where a drug intervention might be efficacious and specific have highlighted the need for an accurate and comprehensive catalog of the molecular specifics of the TLR pathways (Li et al. 2009; Oda and Kitano 2006). Which

proteins and small molecules participate in these processes and what are the regulatory events that modulate them? What complexes do the participating molecules form? What biochemical reactions do the proteins carry out? How are these processes compartmentalized within a cell? To what extent are these entities and events involved in other biological processes? While humans are the target of clinical interventions, TLR proteins are well conserved over vertebrate lineages (e.g., Cormican et al. 2009; Roach et al. 2005) and some data concerning TLR function derive from studies of model systems. How can data from these diverse systems be integrated to provide a reliable description of human TLR function?

Pathway databases are useful tools for collecting and integrating data, systematically associating the molecular specifics (proteins, small molecules, and complexes) with the processes and regulatory events that modulate those molecular specifics. Each step of a process is described by a reaction; by linking reactions into pathways by means of shared molecules, complex processes are cataloged. Here we describe one such database, Reactome (Matthews et al. 2009; Vastrik et al. 2007), and its use to annotate species-specific TLR function.

The Reactome data model represents biological processes as reactions that convert input physical entities into output physical entities (Fig. 1a). Each reaction is located in a subcellular compartment and is mediated by the action of other physical entities acting as catalysts and positive or negative regulators. Data are segregated by species. Reactome captures physical entities and their interactions in a frame-based data model. Classes (frames) describe concepts such as reaction, physical entity, subcellular location, and catalysis. Class attributes hold specific identifying information about instances.

The reaction class attributes include the reactants (input), products (output), catalyst, and the subcellular location. Attributes of a catalyst instance are a physical entity and a Gene Ontology (GO) (Ashburner et al. 2000; Gene Ontology Consortium 2010) Molecular Function term that describes the activity of the catalyst. Instances of the regulation class link reactions to the factors that modulate them. By extending the concept of a biochemical reaction to include events such as the association of molecules to form a complex, the transport of a molecule between two cell compartments, and the transduction of a signal, the full range of processes relevant to signal initiation, propagation, and action on a target can be expressed in a single, internally consistent format (Fig. 1b).

Physical entities include proteins, nucleic acids, and small molecules. Proteins and polynucleotides can undergo conformational changes, be modified, moved from place to place, and cleaved. This information is captured by treating the same protein in distinct compartments or with different modifications as a separate physical entity. The modification process itself is a reaction in which the input is the unmodified physical entity and the output is the modified one. Chemically identical entities located in different compartments are represented as distinct physical entities. Transport events are therefore ordinary reactions in which a molecule moves from one distinct compartment to another.

The annotation of alternative locations, post-translational modifications, and conformations of a molecule causes instances of a physical entity to proliferate. The basic chemical information that all forms share is stored in a separate class of reference physical entities, reducing error, facilitating data maintenance, and explicitly linking multiple alternative forms of a single entity to the appropriate external reference database: UniProt for proteins (UniProt Consortium 2010), ChEBI for small molecules (de Matos et al. 2010), and EMBL for nucleic acids (Leinonen et al. 2010).

Many biological reactions involve macromolecular complexes. The Reactome Knowledgebase annotates these entities as instances of the complex class, the attributes of which are subcellular location and the identities of the complex's components. Modified or unmodified macro-molecules, small molecules, and other complexes all can be components of a complex. Molecular assembly is described as a reaction with the separate complex components as input and the assembled complex as output.

Reactions in the Reactome Knowledgebase are supported by direct or indirect evidence. Direct evidence for a human reaction comes from an assay on human cells described in a research publication whose PubMed identifier is stored as an attribute of the reaction. Much biomedical knowledge, however, derives from studies of nonhuman systems thought to be good functional homologs of human ones. Such nonhuman data are used to document a human reaction in two steps. First, we annotate the reaction in the nonhuman species using the physical entities of that organism and cite appropriate literature references. Second, we annotate the human reaction using human physical entities. The human reaction is flagged to indicate its inference from the model organism reaction and, if appropriate, a literature reference is attached that provides evidence that the process is well-conserved and hence that the inference is likely to be valid. The complete chain of evidence is preserved from the primary experiment to the nonhuman reaction to the inferred human reaction.

This process of manual annotation and manual inference of reactions and pathways is labor-intensive. Genome sequencing projects have enabled the predictions of proteomes for diverse species and have revealed extensive protein sequence conservation among species. At the same time, many human processes cannot be studied in vivo but must be approached indirectly via studies of model systems. Can these predicted protein sets and functions for multiple species be integrated with a curated reaction set to enable reliable inference of reactions across species?

Here, we use TLR signaling in human and chicken as a case study to investigate the process of predicting reaction sets for a less well-studied organism from curated data for a well-studied one. Starting with the previously annotated human TLR signaling processes, we describe two approaches to the annotation of chicken TLR signaling, one a continuation of the manual annotation process and the second carried out computationally, applying an OrthoMCL strategy (Li et al. 2003) to infer chicken processes based on curated human ones and the set of chicken proteins predicted from the chicken genome sequence.

Materials and methods

The manually curated human Reactome data set, assembled as described by Vastrik et al. (2007), includes annotations for ligand binding by TLR2, 3, 4, 5, 7, 8, 9, and 10 and downstream signaling via MyD88, TRIF, and TRAM cascades, supported by evidence from studies of human proteins and cells (http://www.reactome.org/cgi-bin/event browser?DB=gk_current&ID=168898&). We have now assembled a manually curated chicken data set that includes annotations for ligand binding by TLR2, 3, 4, 5, 7, 15, and 21 and downstream signaling via MyD88, TRIF, TRAM, and NF- κ B, supported partly by data from studies of chicken proteins and cells and partly by manual inference from properties of homologous human proteins. In parallel, the OrthoMCL (Chen et al. 2006; Li et al. 2003) inference strategy was applied to the ENSEMBL release 53 pep.all data set (ftp://ftp.ensembl.org/pub/release-53/fasta/gallus_gallus/pep/) to predict chicken TLR reactions based on the manually curated human ones as described previously (Vastrik et al. 2007).

Unrooted trees for chicken and human Toll-like receptors were generated in two steps. First, multiple sequence alignments for the sequences from each species were performed separately with CLUSTAL 2.0.12 using default parameters. Unrooted phylograms were then generated for the two alignments with the PyIip Neighbor Join algorithm using the Jones-Taylor-Thorton distance matrix model.

Results

Previous analyses of chicken EST and genomic DNA sequences have identified ten expressed TLR genes and defined the phylogenetic relationships between those genes and the human expressed TLR genes. Single genes orthologous to human *TLR3*, *-4*, *-5*, and *-7* have been identified. The *TLR2* gene present in a single copy in the human genome is found as a tandem array of two expressed genes in the chicken. The chicken *TLR1LA* and *TLR1LB* genes, also present as a tandem array, derive from a common ancestor of the human *TLR1*, *-6*, and *-10* genes. Unrooted phylogenetic trees for these human and chicken TLR proteins are shown in Fig. 2.

Like human TLR2 proteins, chicken TLR1LA and TLR1LB have been shown to form functional heterodimers with either chicken TLR2 protein; ligand specificity is dependent on the TLR heterodimer composition (Higuchi et al. 2008; Keesstra et al. 2007). Finally, no chicken genes capable of encoding orthologs of human TLR8 or 9 have been found, while the chicken genome but not the human one encodes TLR15 and 21 (Cormican et al. 2009; Roach et al. 2005; Temperley et al. 2008; Yilmaz et al. 2005).

Hundreds of human gene products have been associated with innate immune function. Analyses of the chicken genome sequence and EST collections together with high-throughput expression studies have identified putative homologs of a majority of these (Kaiser et al. 2008; Lynn et al. 2003; Temperley et al. 2008) and specific ligands for some of them have been identified (Table 1). The aims of this study were to ask whether the orthology analysis of TLR proteins could be extended to the other downstream components of the TLR signaling pathways, whether functional relationships between human and chicken inferred from sequence similarities could be reinforced by comparative annotation of protein function in a pathway context, and to estimate the extent to which reliable functional annotation could be generated computationally based on an OrthoMCL strategy for identification of orthologs. Here we focus specifically on signaling processes initiated by the TLR3 receptor, a process that has been analyzed experimentally in considerable detail in humans but only to a limited extent in the chicken (Fig. 1c).

TLR3 plays a vital role in host responses to viral double-stranded RNA. Mammalian TLR3 activated by ligand binding recruits TRIF, with three known consequences (Wheaton et al. 2007). The first is activation of interferon regulatory factor 3 or 7 (IRF3/7) mediated by IKK-related kinases. Activated IRF3 or 7 mediates innate antiviral responses through interferon- α and - β expression. The second is RIP1 recruitment followed by activation of NF- κ B mediated by I κ B kinase (IKK). The third is TRAF6 (tumor necrosis factor receptor-associated factor 6) activation, which in turn induces phosphorylation of TGF- β -activating kinase 1 (TAK1). Phosphorylated TAK1 can mediate the induction of the transcription factor NF- κ B or mitogen-activated protein kinases (MAPKs) such as JNK or p38. This results in the translocation of the activated NF- κ B and MAPKs to the nucleus and transcriptional activation of genes encoding proinflammatory cytokines and antimicrobial peptides. These human proteins, the complexes formed, and other proteins with which they interact in these signaling cascades are listed in Table 2.

The chicken TLR3 signaling cascade has not been systematically characterized. Experimental data for individual proteins together with orthology-based inferences allow its manual reconstruction, however, as shown in Fig. 1c and the “curated *Gallus gallus*” section of Table 1. Comparison of the genomic sequences of human and chicken has confirmed the identification of a single chicken TLR3 protein 61% identical to its human ortholog (Roach et al. 2005; Temperley et al. 2008). The ability of human HEK293 cells transfected with the chicken *TLR3* gene to respond to polyI:C in a standard reporter assay provides evidence of conserved function (Schwarz et al. 2007). It was also shown in the chicken, as in mammals, that polyI:C rapidly induces expression of type 1 interferon and TLR3 (Karpala et al. 2008).

TAK1 function in chickens has not been linked experimentally to innate immune responses, but its kinase activity has been characterized and shown to be essential for B-cell antigen receptor-mediated IKK and JNK activation in chicken DT40 B cells (Shinohara et al 2005). B-cell antigen receptor-mediated NF- κ B activation was abolished and JNK phosphorylation was completely abolished, while ERK was activated normally in TAK1-deficient chicken DT40 B-cell line.

Phylogenetic analyses with the OrthoMCL tool identified chicken orthologs for most of the other proteins identified in studies of human TLR3 signaling (Table 2). Notable exceptions were IKK γ , MKK7, and IRF3 (Fig. 1c, blue highlighting), for which no orthologs could be identified, and human *MKK3* and *MKK6* genes, which both best matched the single chicken gene ENSGALG00000004735.

Through this combination of direct experimental evidence from chicken systems and inference by orthology, we manually identified chicken counterparts of 33 of the 38 proteins in the annotated human TLR3 pathway (Table 2; Fig. 1c, red highlighting). When the fully automated OrthoMCL inference strategy (Li et al. 2003; Vastrik et al. 2007) was applied to these same human data, chicken counterparts for 29 of the human proteins were identified.

The automated approach yielded no false-positive identifications (Table 2, compare “curated” and “inferred” *Gallus gallus* columns).

Discussion

Reactome is not the only readily available pathway data-base. Others with extensive data relevant to signaling processes include KEGG (Kanehisa et al. 2010), MetaCyc (Caspi et al. 2010), the NCI-Nature Pathway Interaction Database (Schaefer et al. 2009), and PANTHER (Thomas et al. 2003). The Reactome data model and curation process, however, makes it particularly well suited for the process of annotating a molecular pathway in a reference species in which it has been well characterized experimentally and then projecting those annotations onto other less well-studied species.

The Reactome data set is manually curated to use only data from a single species to construct a biological pathway. This method of manual curation provides a platform for the comparison of computational projections with manual pathway curation.

For the TLR3 signaling pathway, we present a manual curation strategy that leverages the speed of the computational projection with the fidelity of manual curation. Using the computational projection from human to chicken as a starting point, a curator was able to extend our annotation of the chicken pathway beyond that of the computational prediction and to refine the predictions made. Such a curatorial loop allows us to identify key species differences in core biological processes; a schedule of annotation revision allows us to keep the material current.

The failure to observe a chicken ortholog of human IKK γ is striking but must be interpreted with great caution as the current version of the chicken genome sequence is not complete and may not be completely correctly assembled.

A concrete knowledge of the functional biological differences between model organisms is critical if researchers are to correctly interpret their experimental results and extend their finding in a model organism back to humans. In the case of TLR signaling, it is notable that despite the differences in their TLR protein families, chicken heterophils and spleen cells are responsive to the broad range of known mammalian TLR antagonists, including ligands that stimulate mammalian TLR7/8 and TLR9 (He et al. 2006,2007; Kogut et al. 2005,2007;Schwarz et al. 2007). It will be of considerable interest to trace the basis of the individual molecular functions that enable these structurally divergent TLR sets to mediate parallel responses to microbial threats.

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Fig. 1.

TLR3 signaling pathways in human and chicken. a A generic reaction diagram with two inputs, one catalyst, and one output physical entities. b Generic reaction diagrams illustrating prototypical binding, cleavage, modification, and transport reactions. c Diagrammatic view of parts of the TLR3 signaling pathways in *Gallus gallus* and *Homo sapiens*. Pathways have been manually laid out for ease of comparison. *Gallus gallus* entities that were identified by manual curation but were not identified with the OrthoMCL computational prediction are highlighted in red. *Homo sapiens* entities and complexes that do not appear in the *Gallus gallus* set are highlighted in blue. Table 2 contains a complete list of the differences among the manually curated human, manually curated chicken, and computationally inferred chicken TLR3 pathways (Color figure online)

Fig. 2.

Unrooted trees for chicken and human Toll-like receptors. Trees are shown without distance units. a Chicken: TLR2 type 1 (UniProt: Q9DD78), TLR1-1 (UniProt:Q5WA51), TLR1-2 (UniProt:A5H0S2), TLR5 (UniProt:Q5GR02), TLR4 (UniProt:Q7ZTG5), TLR3 (UniProt:Q0PQ88), TLR15 (UniProt:Q2XQ10), TLR21 (UniProt:Q5ZJ34), TLR2 type 2 (UniProt:Q9DGB6), TLR7 (UniProt:Q5ZJD0), TLR2 (UniProt:O60603), and TLR3 (UniProt:O15455). b Human: TLR2 (UniProt:O60603), TLR3 (UniProt:O15455), TLR4 (UniProt: O00206), TLR5 (UniProt:O60602), TLR6 (UniProt:Q9Y2C9), TLR8 (UniProt:Q9NR97), TLR10 (UniProt:Q9BXR5), TLR1 (UniProt: Q5FWG5), TLR9 (UniProt:Q9NR96), TLR7 (UniProt:Q9NYK1)

Known chicken TLRs and their ligands

Table 1

Name	Ligand	Pathogen	Gene ID ^a	Reference
TLR1/1LA	Lipoproteins	Bacteria	017485	Higuchi et al. 2008; Keestra et al. 2007
TLR1/1LB	Lipoproteins and peptidoglycans	Bacteria	EntrezGene 771173	Higuchi et al. 2008; Keestra et al. 2007
TLR2A	Lipoproteins and peptidoglycans	Bacteria	009237	Higuchi et al. 2008; Keestra et al. 2007
TLR2B	Lipoproteins and peptidoglycans	Bacteria	009239	Higuchi et al. 2008; Keestra et al. 2007
TLR3	dsRNA	Viruses	013468	Schwarz et al. 2007; Karpala et al. 2008
TLR4	LPS	Bacteria	007001	Kogut et al. 2005; Keestra and van Putten 2008
TLR5	Flagelin	Bacteria	009392	Iqbal et al. 2005; Keestra et al. 2008
TLR7	ssRNA, imidazoquinoline compounds	Viruses	016590	Philbin et al. 2005
TLR15	Unknown	Bacteria	008166	Higgs et al. 2006; Nerren et al. 2010
TLR21	CpG oligodeoxynucleotides	Bacteria and viruses	000774	Brownlie et al. 2009

^aWhenever possible, genes are identified by Ensembl IDs. Only the last six digits of each ID are shown, e.g., ENSG00000000701 is represented as 00701. In the one case where no Ensembl ID was available, the EntrezGene ID was used for identification

Table 2

Differences between curated components of the human TLR3 signaling pathway and their chicken counterparts, manually and computationally derived

Complex/set name	Curated <i>Homo sapiens</i>		Curated <i>Gallus gallus</i>		Inferred <i>Gallus gallus</i>	
	Component name	Gene ID ^a	Component name	Gene ID ^b	Component name	Gene ID
IKK complex (IKKc)	IKK1	213341	IKKA (IKK1)	003289	[not found]	
	IKK2	104365	IKKB (IKK2)	003740	[not found]	
	IKKG	073009	[not found]		[not found]	
Activated IKK complex (IKK+)	Phospho-IKK1 (pIKK1)	213341	pIKKA	003289	[not found]	
	Phospho-IKKB (pIKKB)	104365	pIKKB	003740	[not found]	
	IKKG	073009	[not found]		[not found]	
IKBs: NF-κB complex (IKB: NFKB)	IKBA	100906	IKB alpha (IKBA)	010063	[not found]	
	IKBB	104825	[not found]		[not found]	
	REL A	173039	REL A	EntrezGene 396027	[not found]	
Phospho-NF-κB inhibitor (pIKB)	NFKB1 p50	109320	NFKB1 p50	012304	[not found]	
	NFKB2	077150	[not found]		[not found]	
	Phospho-IkBA (pIkBA)	100906	pIkBA	010063	[not found]	
NF-κB complex (NFKBc)	Phospho-IkBB (pIkBB)	104825	[not found]		[not found]	
	REL A	173039	REL A	EntrezGene 396027	REL A	007845
	NFKB1 p50	109320	NFKB1 p50	012304	NFKB1 p50	012304
IRF3/IRF7 (IRF3,7)	NFKB2	077150	[not found]		ENSGALP000000020084	012304
	IRF3	126456	[not found]		[not found]	
	IRF7	185507	IRF7	014297	IRF7	014297
Phosphorylated IRF3/IRF7 (pIRF3,7)	Phospho-IRF3	126456	[not found]		[not found]	
	Phospho-IRF7	185507	pIRF7	014297	pIRF7	014297
MKK4/MKK7 (MKK4,7)	MKK4	065559	MKK4	001001	ENSGALP000000001475	001001
	MKK7	076984	[not found]		[not found]	
	Phospho-MKK4/phospho-MKK7	065559	pMKK4	001001	pSEK1	001001
MKK3/MKK6 (MKK3,6)	Phospho-MKK7 (pMKK7)	076984	[not found]		[not found]	
	MKK3	034152	MKK3/MKK6	004735	MKK3/MKK6	004735
	MKK6	108984				

Complex/set name	Curated <i>Homo sapiens</i>		Curated <i>Gallus gallus</i>		Inferred <i>Gallus gallus</i>	
	Component name	Gene ID ^a	Component name	Gene ID ^b	Component name	Gene ID
Phospho-MKK3:phospho-MKK6 (pMKK3;pMKK6)	Phospho-MKK3 (pMKK3)	034152	pMKK3/MKK6	004735	pMKK3/MKK6	004735
	Phospho-MKK6 (pMKK6)	108984				
JNKs: MAPK8 or MAPK9 or MAPK10 (JNKs)	JNK1 (MAPK8)	107643	JNK1(MAPK8)	006109	JNK1(MAPK8)	013761, 011109, 006109
	JNK2 (MAPK9)	050748	JNK2(MAPK9)	013761	JNK2(MAPK9)	013761, 011109, 006109
	JNK3 (MAPK10)	109339	JNK3(MAPK10)	011109	JNK3(MAPK10)	013761, 011109, 006109
Phosphorylated JNKs: MAPK8 or MAPK9 or MAPK10 (pJNKs)	Phospho-JNK1 (MAPK8) (pJNK1)	107643	pJNK1(MAPK8)	006109	pJNK1(MAPK8)	013761, 011109, 006109
	Phospho-JNK2 (MAPK9) (pJNK2)	050748	pJNK2(MAPK9)	013761	pJNK2(MAPK9)	013761, 011109, 006109
	Phospho-JNK3 (MAPK10) (pJNK3)	109339	pJNK3(MAPK10)	011109	pJNK3(MAPK10)	013761, 011109, 006109
IKK-related kinases TBK1/IKK epsilon (TBK1,IKKE)	TBK1	183735	TBK1	009840	TBK1	009840, 013356
	IKKE	143466	IKKE	013356	IKKE	013356, 009840
Viral dsRNA: TLR3:TRIF:phospho-TBK	Viral dsRNA (-) stranded		Viral dsRNA (-) stranded		Viral dsRNA (-) stranded	
Phospho-IKKE complex (vRNA complex)	TLR3	164342	TLR3	013468	TLR3	013468
	TRIF	127666	TRIF (TIKAM1)	024109	TRIF	024109
p38 MAPK : MAPKAPK2 or MAPKAPK3 (MAPK:MAPKAPc)	TBK1	183735	TBK1	009840	TBK1	009840, 013356
	IKKE	143466	IKKE	013356	IKKE	013356, 009840
	MAP kinase p38 β isoform 1 (MAPK14)	185386	MAPK14	019759	[not found]	
	MAP kinase p38 α (MAPK11)	112062	MAPK11	008612	MAPK11	019384, 019759, 008612
Phospho-p38 MAPK:MAPKAPK2 or	MAPKAPK2	162889	MAPAPK2	000883	MAPKAPK2	000883, 002283
	MAPKAPK3	114738	MAPKAPK3	002283	[not found]	
Phospho-p38 MAPK:MAPKAPK2 or			MAPK12	019384	[not found]	
			MAPK13	000826	[not found]	
	Phospho-MAP kinase p38 β isoform 1 (MAPK14)	185386	pMAPK14	019759	[not found]	
	Phospho-MAP kinase p38 α (MAPK11)	112062	pMAPK11	008612	pMAPK11	019384, 019759, 008612
MAPKAPK3 (pMAPK:MAPKAPc)	MAPKAPK2	162889	MAPAPK2	000883	MAPKAPK2	000883, 002283
	MAPKAPK3	114738	MAPKAPK3	002283	[not found]	

Complex/set name	Curated <i>Homo sapiens</i>		Curated <i>Gallus gallus</i>		Inferred <i>Gallus gallus</i>	
	Component name	Gene ID ^a	Component name	Gene ID ^b	Component name	Gene ID
Phospho-p38 MAPK:phospho-MAPKAPK2 or (MAPK14)	Phospho-MAP kinase p38 β isoform 1	185386	MAPK12	019384	[not found]	
			pMAPK13	000826	[not found]	
			pMAPK14	019759	[not found]	
Phospho-MAPKAPK3 (pMAPK;pMAPKAPc)	Phospho-MAP kinase p38 α (MAPK11)	112062	pMAPK11	008612	pMAPK11	019384, 019759, 008612
	Phospho-MAPKAPK2 (pMAPKAP2)	162889	pMAPAPK2	000883	pMAPKAPK2	000883, 002283
	Phospho-MAPKAPK3 (pMAPKAP3)	114738	pMAPKAPK3	002283	pMAPKAPK3	000883, 002283
			pMAPK12	019384	[not found]	
MEK1:ERK-1	Phospho-MEK1 (pMEK1)	169032	pMEK1	007687	pMEK1 homolog	001267, 007687
	ERK1	102882	ERK1	hypothetical	ERK1	001501
Phospho-ERK1:MEK1 (pERK1:MEK1)	Phospho-MEK1 (pMEK1)	169032	pMEK1	007687	pMEK1 homolog	001267, 007687
	Phospho-ERK1 (pERK1)	102882	pERK1	hypothetical	pERK1	001501
MEK2:ERK-2	Phospho-MEK2 (pMEK2)	126934	pMEK2	001267	pMEK2	001267, 007687
	ERK2	100030	ERK2	001501	ERK-2	001501
Phospho-ERK2:MEK2 (pERK2:MEK2)	Phospho-MEK2 (pMEK2)	126934	pMEK2	001267	pMEK2	001267, 007687
	Phospho-ERK2 (pERK2)	100030	pERK2	001501	pERK2	001501

Abbreviated names of complexes and components used in Fig. 1C are shown in parentheses: p = phospho; c = complex, + = activated

^a Genes are identified by Ensembl IDs. Only the last six digits of each ID are shown, e.g., ENSG00000100030 is represented as 100030

^b Genes are identified by Ensembl IDs unless otherwise noted. Only the last six digits of each ID are shown, e.g., ENSGALG00000001501 is represented as 001501