

# A Graphical Notation to Describe the Logical Interactions of Biological Pathways

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## Summary

The modelling of biological intra-cellular pathways requires the systematic capture and representation of interactions between components that are biologically correct and computationally rigorous. The challenge is two fold, to verify and extend our understanding of such pathways by comparing *in silico* models to experiments; and to ensure that such models are understandable by biologists and for checking biological validity. In this report we present a graphical notation, the Edinburgh Pathway Notation (EPN), which satisfies the central biologically driven requirements while providing a strict formal framework for analysis. The EPN emphasises the use of a logical representation of pathways, which is particularly suited to pathways where some mechanisms are not known in detail.

## 1 Introduction

The challenge in Systems Biology is to find way of assimilating the vast amount of information in the literature and experimental data that provide an understanding of how biological pathways work at the molecular level within a cell and at the organism or tissue level. In order to test our understanding it is necessary to develop models of such pathways that we can compare to experimental observation. This requires that we first have a mechanism for building such models, and second, that our model reflects current biological understanding [1-4]. The approach taken in this paper and by others is to use a graphical notation that describes a pathway visually, but which is sufficiently detailed and unambiguous that a computational model can be derived from it [5-10].

Informal graphical descriptions of biological pathways are commonly used in reviews and papers. They are extremely useful aids to understanding, but are not sufficient by themselves and typically rely on some supplementary text to describe the details of the pathway's behaviour. Since such prose is inherently ambiguous [11] these diagrams are not optimal for model building. Given the apparent resemblance between complex biological pathways and certain aspects of electronic circuits there is often a naïve assumption that the same modelling techniques can be applied to biological systems. However, the products of engineering design are inherently man made, and therefore adhere to a defined set of rules, which have well understood boundaries and interfaces. In contrast our knowledge and understanding of biological systems are by definition incomplete. This means that we cannot be sure we know

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all the rules that apply to a system, nor the boundaries of the system. Accordingly, biology-specific approaches are called for.

To date two types of representation, entity-relationship and state transition, have so far been employed to describe biological pathways. The first type describes the components in a pathway and how they interact with each other. Interactions are typically described as relationships between entities. To describe a biological pathway proteins and genes are shown only once and their interactions are typically described by connecting lines and arrows. This results in a relatively compact notation at the expense of increased complexity for the reader. Examples are the Molecular Interaction Map (MIM) [12, 13] and Kitano block diagrams [6]. The second type of representation is the state-transition diagram. Here the states of an entity is described explicitly (rather than implied as in the entity relationship view) and a biochemical reaction is represented as a conversion of one or more entities from one set of states to another (a state transition). The Process Description Notation (PDN) [6, 7] is the most successful example of a state-transition based graphical notation. The PDN is simple to understand and its use is relatively easy thanks to its supporting pathway modelling software, CellDesigner ([www.celldesigner.org](http://www.celldesigner.org)) [5]. A potential limitation of the state-transition representation is that it can be susceptible to a combinatorial explosion of states if there are a large number of state modifiers that can combine with each other [14]. This may well be a problem for key regulatory proteins such as p53, which has multiple chemical modification sites and which interacts with number of proteins [15, 16].

Orthogonal to these representations are the biochemical and logical views of a pathway. Both the PDN and MIM describe a pathway as a set of biochemical reactions, but it is also possible to describe a pathway in a logical manner [17]. A common example is a biologist describing a protein as “activated”. Biochemically, this may mean that the protein is phosphorylated, but logically it means that it is a state that enables it to initiate some other action in the pathway. The power of this representation comes when the biochemical detail is unknown. The biologist may know the protein is “activated” and the consequences of that activation, but not know how it occurs in biochemical detail.

Here we present, the Edinburgh Pathway Notation (EPN), which uses a logical state-transition representation to describe biological pathways. EPN fulfils the objective of providing a graphical notation that is both useable by biologists and which can serve as the basis for computational model development.

## 2 Methods

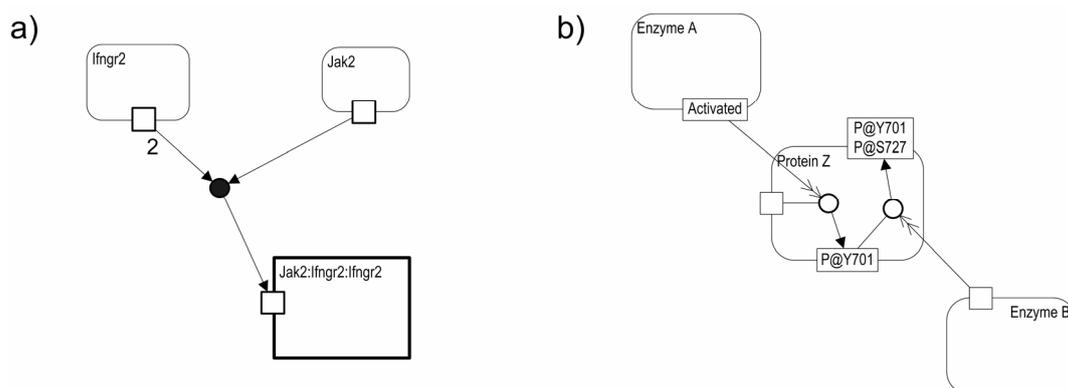
We sought to develop a graphical notation that fulfils the following criteria: **1) The notation should be useable by biologists.** This means that they should be able to understand it easily and that they should be able to create models using it with minimal training. This is important because the notation should be a tool for communication and unless it is understandable pathways described using the notation would be very hard to validate for biological correctness. **2) The notation should be computable.** That means it should be relatively easy to develop drawing software that supports this notation and that it should be possible to create *in silico* models of the pathways described by the notation. In the latter case models ideally should be written using SBML [18] or another mark up language to represent biological models. **3) The notation should be compact.** By compact we mean taking up as little drawing space as possible, while remaining expressive and legible. Biological pathways can be very large and once the size of a diagram extends over a single screen or page this “fragmentation” becomes a barrier to comprehension. Therefore, economical use of space in a pathway map allows larger pathways to be viewed and assists a user’s assimilation. **4) Show subcellular localisation.** When modelling a biological pathway in eukaryotic cells it is

crucial to know where in the cell an interaction occurs. For example a gene regulatory protein cannot regulate a gene unless it is in the nucleus of a cell. Indeed the regulation of the translocation of proteins or complexes is an important mechanism in pathway control [19, 20].

**5) Tolerance of incomplete knowledge.** One of the biggest challenges to developing models of biological pathways is that our knowledge is incomplete. Any notation that maps biological pathways must therefore accommodate parts of a pathway where the details are only partially or completely unknown.

Through an iterative process of implementing a series of notations with biologists, we evolved a simple notation that focused on the logical description of the pathway, rather than a detailed biochemical description, but which clearly described the state of the interacting entities in the pathway. In order to keep the notation compact we tried to minimise the duplication of entities within the diagrams and to achieve simplicity we have tried to minimise the number of symbols used. The notation itself describes a pathway as a set of state transitions and in it we aim to show clearly when protein species participate in a complex and in which subcellular compartment they are located. Other properties that can describe the state of an entity in a pathway such as chemical modification, conformational change, and gene activation, are shown descriptively rather than with a detailed biochemical representation. Likewise the logic governing the control of processes such as catalysis or gene regulation is shown using Boolean logic gates. This is particularly useful when describing gene regulation since it allows us to ignore the detailed biochemical mechanisms of gene regulation and focus on the gene regulatory components and the logic of how they regulate the gene.

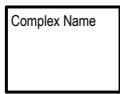
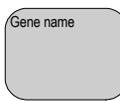
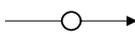
The notation describes biological pathways as a series of related state transitions. Each entity within the pathway can exist in one or more states which relate to biological phenomenon, for example chemical modification (phosphorylation) or conformational change. If the entity can adopt another state the conversion from the first state to the next is described as a state transition. In addition a state transition can be activated by another entity in a given state. Processes such as complex formation, complex dissociation, protein cleavage and subcellular translocation are all regarded as special types of state transition. By combining these concepts it is possible to describe biological processes as shown in figure 1.



**Figure 1: Examples of the notation in use. a) shows complex formation. The Jak2 and two copies of Ifngr2 form a complex. b) Phosphorylation of Protein Z is shown in terms of state transition.**

In order to provide a more fine grained description of pathways we use Boolean logic operators to describe the activation of state transitions. The operators provided are AND, OR, XOR and NOT and they are particularly useful for describing gene regulation (figure 2). Gene regulation requires the introduction of an addition protein expression operator, which results in the creation of a new entity. The state based description is useful when describing gene regulation, especially when a gene can be regulated at multiple regulatory sites. In this case each regulatory mechanism can be represented as a separate gene state and the logic that

controls that state can be clearly shown. The complete set of symbols used in the notation can be seen in table 1 and the rules about how they can be connected are shown in table 2.

| Symbol  | Name               | Description   | Symbol  | Name       | Description  |
|---|--------------------|---|---|------------|--|
|    | Complex            | A protein complex. This can be a homo or hetero - dimer.  |    | Cleavage   | Cleavage of a protein into two or more proteins.   |
|    | Protein            | A monomeric protein.  |    | State      | The state of a complex or protein. If unlabeled the default or baseline state is indicated. The state name should be descriptive and unique to the particular species. |
|    | Gene               | A gene.   |    | Activate   | Initiates of a state transition.   |
|    | State transition   | An action that transforms a species from one state into another. Examples are a chemical modification or conformational change.   |    | Inhibition | Causes a state transition not to occur.  |
|  | Complex formation  | Two or more proteins (or complexes) aggregating to form a complex.  |  | AND        | Boolean AND gate.  |
|  | Dissociation       | A complex breaking apart to form two or more proteins or complexes.   |  | OR         | Boolean OR gate.   |
|  | Protein expression | Transcription and translation of a gene to generate a gene product.   |  | XOR        | Boolean XOR gate.  |
|  | Translocation      | Movement of a protein or complex from one subcellular location to another. This is a type of state transition and can be activated or inhibited in the same way as other state transitions. |  | NOT        | Boolean NOT gate   |

**Table 1: Symbols used in the Edinburgh Pathway Notation. The symbol together with the name and its description are shown.**

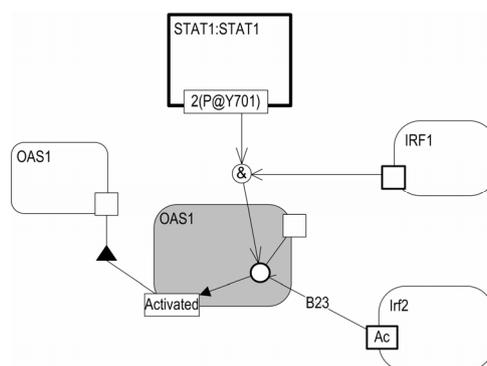
The state of an entity must be unique and, with the exception of the “default” state, it must be named. The default state is a special case that represents the unmodified state of an entity, such as a protein that is not chemically modified, a kinase that has not been activated or a gene that is not being transcribed. Other states should be named - because this is a logical

representation any meaningful name may be used. This gives the notation significant flexibility because it enables biological concepts which are difficult to describe biochemically, such as conformational change, to be represented easily. That said, the notation does allow the user to provide more biochemical detail where it is available by the use of recommended naming conventions for complexes and states. Complexes may be named if the complex itself has a name (often it does not), but in any case the complex should list the subunits that compose the complex. Likewise if a protein is modified chemically, then the state should be named with the standard symbol for the element of chemical group modifying it. This state naming convention can be extended to provide a detailed description of the biochemical modifications of the protein. Where the modified amino acid in the protein is known its code and position should be shown as follows: P@Y701. Multiple sites should be separated by a semi-colon (P@Y701;P@S727) and in a complex the modified subunit should be stated (Stat1:P@Y701;P@S727). Examples are shown in table 3.

|                  | State transition | Complexation | Dissociation | Expression | Translocation | State | Activation | Inhibition | AND | OR | XOR | NOT |
|------------------|------------------|--------------|--------------|------------|---------------|-------|------------|------------|-----|----|-----|-----|
| Complex          |                  |              |              |            |               |       |            |            |     |    |     |     |
| Protein          |                  |              |              |            |               |       |            |            |     |    |     |     |
| Gene             |                  |              |              |            |               |       |            |            |     |    |     |     |
| State transition |                  |              |              |            |               |       |            |            |     |    |     |     |
| Complexation     |                  |              |              |            |               |       |            |            |     |    |     |     |
| Dissociation     |                  |              |              |            |               |       |            |            |     |    |     |     |
| Expression       |                  |              |              |            |               |       |            |            |     |    |     |     |
| Translocation    |                  |              |              |            |               |       |            |            |     |    |     |     |
| State            |                  |              |              |            |               |       |            |            |     |    |     |     |
| Activation       |                  |              |              |            |               |       |            |            |     |    |     |     |
| Inhibition       |                  |              |              |            |               |       |            |            |     |    |     |     |
| AND              |                  |              |              |            |               |       |            |            |     |    |     |     |
| OR               |                  |              |              |            |               |       |            |            |     |    |     |     |
| XOR              |                  |              |              |            |               |       |            |            |     |    |     |     |
| NOT              |                  |              |              |            |               |       |            |            |     |    |     |     |

**Table 2: The connection rules for the symbols in the EPN. The matrix shows symbols can be connected as a grey square, for example a state transition can be connected to an activation or inhibition arrow, but no other symbol.**

To ensure that the notation can be used consistently and correctly there are a simple set of rules to follow. Each entity can only interact with another entity via a state transition (including special forms such as complex formation, or protein expression). A state transition can be activated by a state and another state transition. In the latter case this means that the execution of the first state transition activates the second. Note that a state transition should at most only have one activation arrow and one inhibition arrow assigned to it. Where more than one state activates a state transition a Boolean gate should be used, for example an OR gate, to ensure only that one source of activation is present. Logic gates can only be linked to states via activation or inhibition arrows.



**Figure 2: Examples of gene regulation. a) The diagram uses Boolean gates to show that both the Stat1 homodimer and IRF1 are required to activate gene expression, but that IRF2 inhibits expression.**

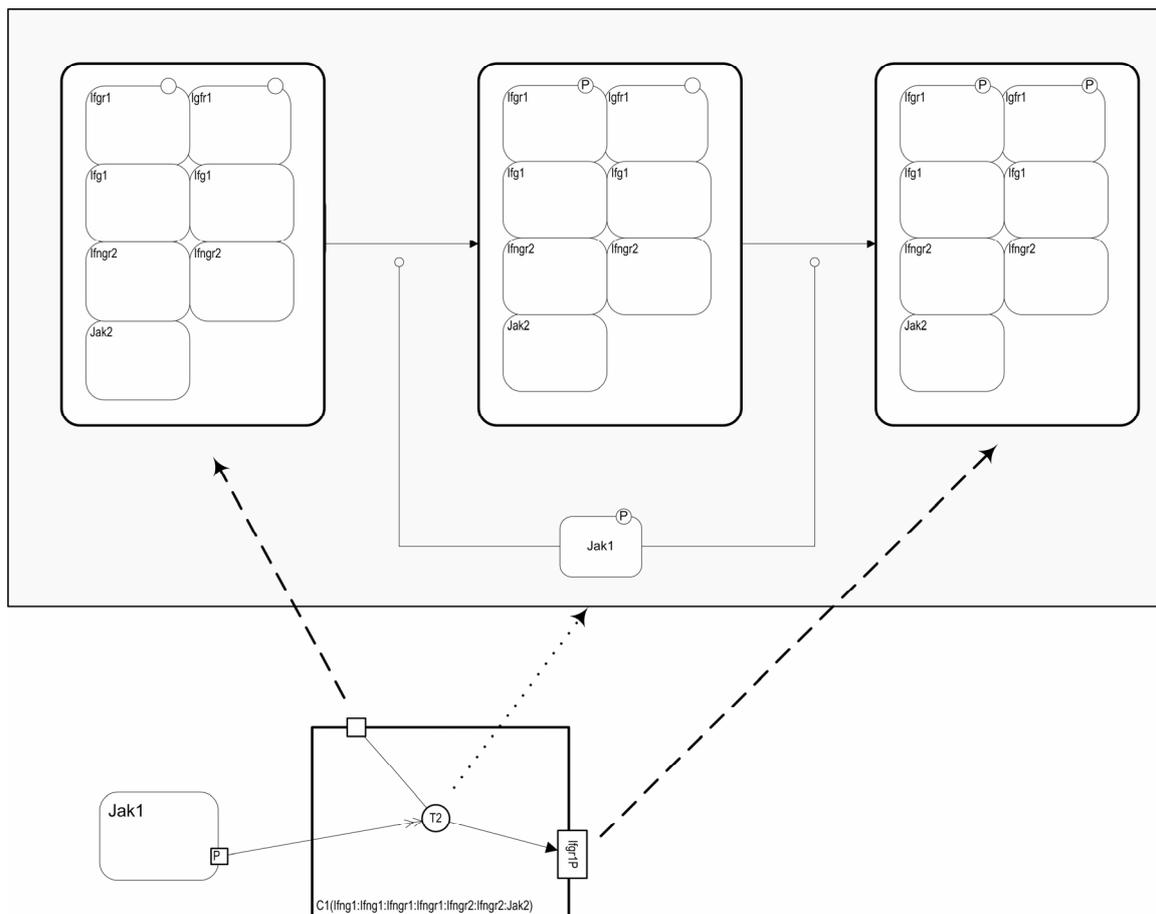
The notation takes a simple approach to representing the location of an entity-state within the cell (subcellular location). It divides the drawing canvas into sections representing each location, with the convention that the top of the canvas is towards the outside of the cell and the bottom the centre, i.e. the nucleus. The author of the diagram is responsible for deciding which compartments to show in the diagram. In order to make the notation as compact as possible an entity of a given species is only shown once in a compartment. In order to show translocation from one compartment to another, entities can be duplicated between compartments. Note that translocation can be shown as a simple arrow, or as a state transition symbol. This is purely for aesthetic reasons. The state transition version allows the regulation of subcellular transportation to be represented, for example by transport proteins in the nuclear membrane.

| Example                                    | Description   |
|--|---|
| GAP(STAT1 $\alpha$ :STAT1 $\alpha$ )       | Complex called GAP which is a STAT homodimer  |
| ISGF3(STAT1 $\alpha$ :STAT2:IRF9)          | Complex called ISGF3 consisting of STAT1 $\alpha$ , STAT2:IRF9  |
| STAT1 $\alpha$ :STAT2                      | STAT1 $\alpha$ , STAT2 heterodimer.   |
| P@Y701                                     | Phosphoylation of a protein at Tyrosine 701. Used when the entity is a protein monomer or a complex containing the same subunits. |
| Stat1 $\alpha$ :P@Y701,P@S727;Stat2:P@Y690 | Phosphorylation of Stat1 at residues Y701 and S727 and Stat2 at Y690.   |
| 2(Stat1 $\alpha$ :P@Y701,P@S727);PIAS:Ub   | Both Stat1 $\alpha$ subunits phosphorylated and PIAS Ubiquitinated.   |

**Table 3: Examples of the naming conventions recommended for complexes and states. Using these conventions it is possible to describe the details of the biochemical reactions represented by the notation.**

The logical representation of the EPN is sufficiently generic to permit it to link to other notations that describe biological pathways in more detail, or which have a different emphasis. We have used the notation to map to the PDN pathway notation and the WIT metabolic notation. We call these subdiagrams, since they represent a level of hierarchical organisation of a pathway map. The key to this mapping is that an EPN state transition must

map to one or more reactions in the subdiagram and the initial and terminal states of the state transition map to the initial and final species in the reaction. Any entity states that activate a state transition should also participate in the reaction. This is illustrated in figure 3.



**Figure 3:** The EPN can be systematically mapped to other graphical notations by applying the rules described in the text. The diagram shows how a state transition can be related to two PDN state transitions and how the EPN states can map directly to the first and last states in the diagram above. Note that the activating species, Jak1, participates in the PDN reactions where it acts as a catalyst. Details of the PDN notation can be found in [7].

An important component of any graphical notation is tool support. Ideally, such a tool will make these diagrams easy to draw, help to enforce its drawing rules and enable the graphical notation to be converted into a computational model. The notation described here has such a tool, the Edinburgh Pathway Editor (EPE; [www.bioinformatics.ed.ac.uk/epe](http://www.bioinformatics.ed.ac.uk/epe); <http://www.research.ibm.com/journal/abstracts/rd/506/sorokin.html>). As well as making the drawing of these models considerably easier, it supports hyperlinks that facilitate the mapping between EPN and other biochemical notations. EPE also allows the exchange of diagrams via its own XML based format, and models derived from the notation can also be exported to an SBML representation [18] that allows models to be created that can be used in pathway simulations and other types of analysis.

### 3 Results

The EPN has been used to describe a number of biological pathways. Evaluation by biologists to date has been very favourable. The notation has been used to develop pathway maps for the Type I and Type II Interferon response, pathway and the extrinsic Apoptosis and NFκB pathways. Feedback from users has indicated that the notation allows the biologist to describe

a pathway very rapidly, is easy to understand and is unambiguous. The notation is used in conjunction with EPE to support the teaching activities here at the University of Edinburgh. An example of the notation describing a biological system is shown in figure 4.

Assessment of the “compactness” of the notation is difficult to measure objectively, but a comparison of a number of small biological pathways using EPN and PDN shows that the EPN uses approximately 30-40% fewer entity-shapes and therefore takes up less page space. The EPN saves space primarily when the pathway has a large number of chemical modifications, because these are shown within the same entity. Pathways where new species are created, for example through complex formation and dissociation or cleavage of a precursor protein, tend to have less of a space saving. We did not compare the notation to MIM maps, but our expectation is that because the MIM does not draw new shapes for complex species that it will take up less space than the EPN.

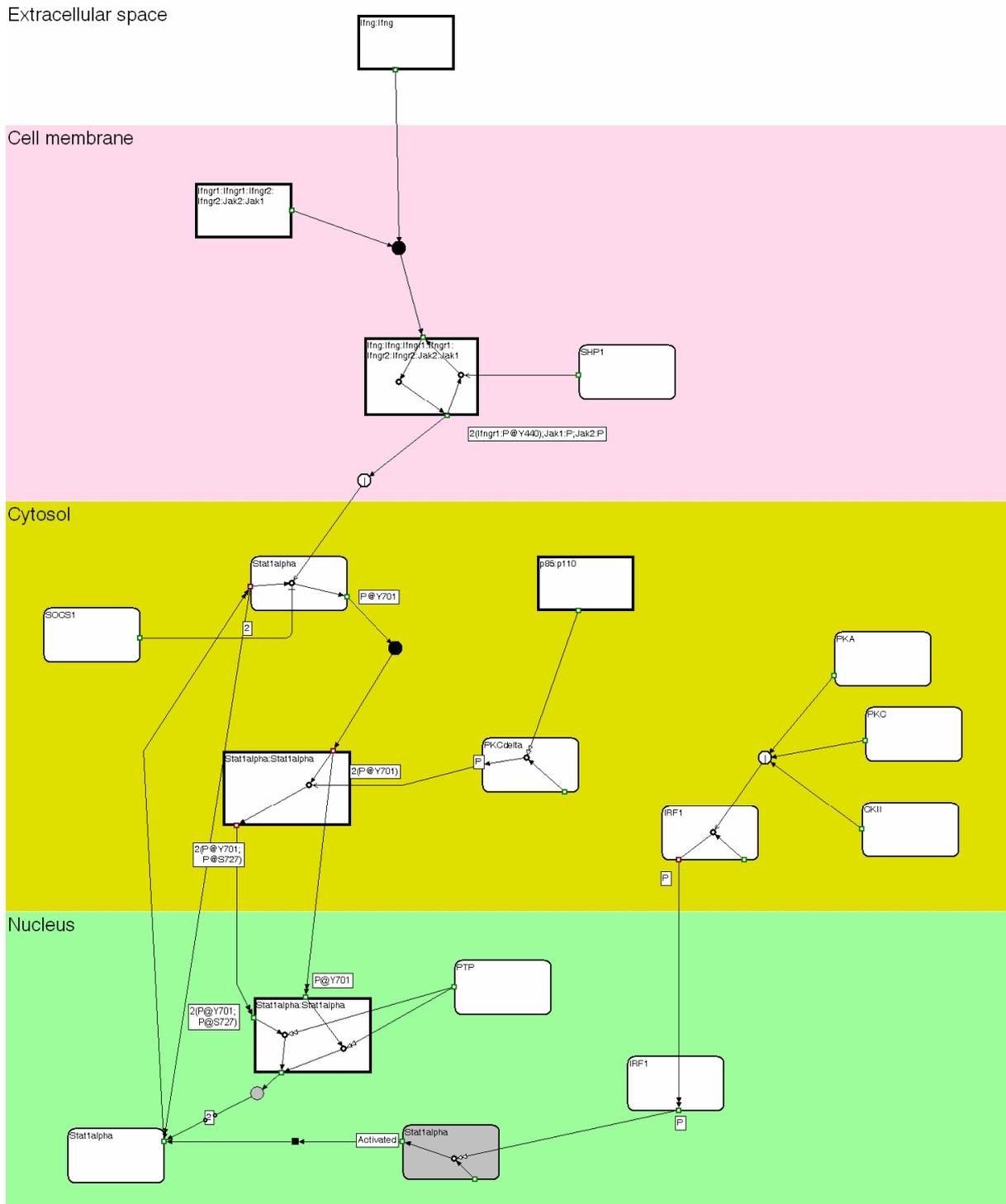
## 4 Discussion

The Edinburgh Pathway Notation takes a different approach to other graphical notations in that it does not provide a biochemical description of a pathway directly. Instead we chose to develop a notation that had a small number of concepts and symbols, but which could cope with biological concepts or biochemical process that have not yet been identified; which would accommodate gaps in our biological understanding of a pathway; and which would enable us to create and biologically validate *in silico* models of these pathways. We feel that when developing a model of a biological pathway a user may want to use a number of types of representations, for example metabolic as well as biochemical. In this case the EPN provides the fulcrum that allows a user to switch from one to the other, but to maintain a mapping between both representations.

In developing and using this notation it is clear there are areas where it could be developed further. The first is to provide a description of mRNA transcriptional regulation, in particular to support differential splicing and events that effect messenger stability, such as micro RNA (miRNA) regulation [21]. It is easy to see how an RNA species could be incorporated into the notation and used in a similar manner to genes and gene regulation. Splice variants could be represented as multiple states and transcript degradation could be modelled by a “degraded” state. A second area for improvement is the representation of species that span subcellular compartments, usually complexes. At present this can be shown explicitly, but can be implied by placing the complex over the subcellular compartment boundary. A better solution is required, possibly by allowing the definition of subcellular compartments within the complex itself.

Perhaps the most significant area of future development is to provide a better way of representing very large pathways maps. Our goal is to eventually have a pathway map for an entire cell, but at present no graphical notation will support such a large map adequately. While the EPN endeavours to be compact and other notations are even more compact (MIM) and or have extensions to make them more succinct (PDN) [7], another approach is required if such maps are to be constructed. Our preferred solution to this problem is to develop a hierarchical representation that shows pathways at different levels of abstraction. This is the approach taken by software modelling languages, such as the Unified Modelling Language (UML; <http://www.uml.org>) or Data Flow Diagrams (DFD) [22]. A related idea, which would assist biological understanding, is to link the pathway descriptions in EPN to cellular states. Currently an EPN map (or a PDN or MIM diagram for that matter) that describes an apoptosis pathway has no way of incorporating an apoptosis event explicitly into the map. Apoptosis tends to be represented by textual annotation. However, apoptosis can be regarded as a cellular state and one can see that if this cellular state could be incorporated into an EPN map

it would provide us with a very convenient way of linking a cellular view of a pathway and its consequences with the detailed molecular view. This is potentially even more powerful if combined with a hierarchical view of a pathway that starts at the cellular level, describing cellular states, and then drills down to the molecular details of the pathway that induces that cellular state. Our intention is to incorporate these ideas into a future version of the EPN.



**Figure 4:** The EPN used to show an extract of the Interferon Type II response pathway incorporating the Jak/Stat pathway. The figure shows the signalling cascade from the phosphorylation of STAT1 $\beta$  in the cytosol, its dimerisation and translocation into the nucleus where it regulates gene expression (not shown to simplify diagram). What can also be seen is the cycling of STAT1 from expression in the nucleus (initiated by activated IRF1), its translocation back into the cytosol, its transport back to the nucleus as a homodimer and then recycling back to the cytosol in its inactive state.

The EPN is a graphical notation that provides a powerful and convenient way of describing biological pathways in a form that is complete, unambiguous and computable. In addition, this notation enables a hierarchical organisation of complementary views of biological pathways by linking logical, biochemical and metabolic views. The notation was used to describe a variety of biological pathways and it has powerful software support to facilitate drawing and *in silico* model creation. This notation was presented at the inaugural Systems Biology Graphical Notation (SBGN) Workshop ([www.sbgm.org](http://www.sbgm.org)) and it is hoped that some of the ideas from this notation will be incorporated into an eventual Systems Biology standard.

## 5 Acknowledgements

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