

**Reasoning from Experiments to Causal Models in
Molecular Cell Biology**

by

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Submitted to the

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in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

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Abstract

This thesis introduces a vocabulary of causal reasoning that is derived from studying experimental biologists. This vocabulary is aimed at formalizing causal language used by biologists, and at capturing causal reasoning methods used by biologists. The thesis introduces a set of causal relations between events; methods for deriving these relations from empirical data; and methods for reasoning with these relations. This theory is embodied in a program that takes as input the raw data from a biology publication, and produces as output a causal model of the system under study. The motivating application for this theory is the construction of an intelligent information retrieval system that could retrieve experimental reports based on queries about causal models. This program has been empirically tested by comparing its performance to the results obtained by experienced biologists working on the same task. This thesis also presents an analysis of the space of causal relations.

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Chapter 1

Introduction

1.1 Motivation

Imagine that it's the year 2001. With the project of determining the structure of the human genome now finished [24], biologists are devoting their efforts to elucidating the causal interactions among the molecular mechanisms found inside cells.

Dr. Joanne Hausdorff has been trying to understand the sequence of events involved in the activation of T-cells in the immune system. In particular, she is trying to understand the interactions between changes in intracellular calcium concentration, and changes in the amount of Interleukin-2 receptors on the surface of a cell. She wonders if there might be a link via Interleukin-2 secretion. She asks her computer:

“Computer, are there any published experiments that show that an increase in calcium concentration causes secretion of Interleukin-2?”

The computer responds:

“Sort of, Dr. Hausdorff. Alemseged Truneh et al. published an experiment in *Nature* (January 1985, volume 313, page 318, figure 3) that showed that an increase in intracellular calcium concentration, *together with* the activation of PKC, caused the secretion of Interleukin-2. An increase in calcium alone did not

cause the secretion of Interleukin-2. Here is the relevant data from that figure:

Additions	IL2 (cpm × 10 ⁻³)
Ionomycin	1
Con A	21
Ionomycin + TPA	22
Con A + TPA	27

Joanne follows up her earlier question:

"Computer, is there any experimental evidence that Interleukin-2 might have an effect on the expression of its own receptor?"

The computer replies:

"Yes, Dr. Hausdorff, Gabrielle Reem and Ning-Hsing Yeh published an experiment in *The Journal of Immunology* (February 1985, volume 134, page 953, table 1) that showed that Interleukin-2 up-regulates the production of its own receptor. Here is the relevant data from that table:

Additions	TAC+ cells (%)	3H-TdR incorp. (cpm × 10 ⁻³)	γ-IFN (U/ml)
—	1	1.0	0.0
Con A	5	11.0	0.8
IL2	1	—	0.0
Con A + IL2	56	300.7	6.0
Con A + Dex	1	0.2	0.2
Con A + IL2 + Dex	26	88.8	6.3
Con A + IL2 + Anti-TAC	20	73.9	2.9

After a few more queries of the computer, Joanne has a good idea of what is known about the system that she is studying. As she turns to the lab bench to try a new experiment, Joanne remembers when it used to take days to perform a literature search, and she marvels at the recent progress in intelligent information retrieval programs.

1.2 Engineering Challenge

The scenario presented in the previous section is not possible today. Today, a biologist using the National Library of Medicine's MEDLINE system can retrieve articles based on keywords. Due to the explosion in the scientific literature—approximately 600,000 biology articles are published annually[2]—keyword-based retrieval has its limits. Keyword searches are not specific enough to find just the right articles. Consider the following statistics:

Search Keyword	Publications Retrieved
Calcium	108,865
Interleukin-2	20,950
T-Cell Activation	5,467

The engineering motivation for this thesis is to lay the foundation for implementing intelligent information retrieval based on searching for experimental evidence for specific causal mechanisms. These systems should provide a vast improvement over current keyword-based retrieval systems.

1.3 Scientific Challenge

We want to understand how people think; reasoning about causality is one of the foundations of human thought.

Although we are most interested in common sense reasoning about causality, there are advantages in studying causal reasoning in a professional, scientific context rather than in an everyday context. One main advantage is that since the objects under study are not directly available for observation, scientists are forced to use indirect observation, and to reason very carefully about the ramifications of their observations. This enforces a certain intellectual hygiene in the reasoning process. Another advantage of studying reasoning in a professional domain is that the demands of publication force scientists to be very explicit and articulate about their observations and conclusions. This guarantees the availability and accessibility of abundant data for building and testing reasoning programs. Finally, since modern experimental biology is a vigorous field making rapid progress, many philosophical

quandries—such as the propriety of reasoning from counterfactuals to causal statements—can be avoided. If an artificial intelligence program models the causal reasoning that biologists perform, the biologists can warrant the utility of the causal reasoning embodied in the program.

1.4 Scenario

The following query can be input into the program described in this thesis. It asks if there are any experiments on T-cells that show IL2 up-regulating (i.e., causing an increase) in the level of TAC stimulated by some cause.

```
(in-system t-cell)
(query (up-regulate il2 ?x tac))
```

The program's response is:

```
(IN-SYSTEM T-CELL)
(REF "Reem and Yeh, J. Immunology, volume 134, page 953, table 1")
(UP-REGULATE IL2 CON-A TAC)
```

This response is based on analyzing the raw data in the biology publication (as shown, for example, in the tables above), and inferring that this data provides experimental evidence that IL2 up-regulates the effect of Con A in causing an increase in TAC measurements.

The program's first step is to analyze the raw data from a biology publication, and to generate qualitative statements about these experimental findings. The program does this by applying simple threshold functions.

For example, the program generates the following qualitative statements about the experimental data presented above:

```
(IN-SYSTEM T-CELL)
(LEADS-TO (CONJ CON-A IL2 DEX) 3H-TDR)
...
(NOT (LEADS-TO IL2 IFN))
...
```

```
(<< (MEASURE 3H-TDR (CONJ CON-A TPA DEX ANTI-TAC))  
      (MEASURE 3H-TDR (CONJ CON-A TPA)))  
(<< 0 (MEASURE 3H-TDR (CONJ CON-A TPA DEX ANTI-TAC)))  
...  
...
```

This intermediate program output expresses the following statements about the experimental results:

- These results are derived from experiments on T-cells.
- It is experimentally observed that causing the conjunction of three events—CON-A, IL2 and DEX—results in the occurrence of the event 3H-TDR.
- Causing the event IL2 does not result in the occurrence of the event IFN.
- The measurement of 3H-TDR in the case where the four events CON-A, TPA, DEX and ANTI-TAC are caused is less than the measurement of 3H-TDR in the case where the two events CON-A and TPA are caused.
- The measurement of 3H-TDR in the case where the four events CON-A, TPA, DEX and ANTI-TAC are caused is greater than zero.

The program's next step is to use these qualitative statements as input to an inference system that generates causal model statements about the biological system under study. For example, the program generates the following causal model statements from the qualitative statements presented above:

```
(IN-SYSTEM T-CELL)  
(ADDITIVE-PARTIAL-BLOCKS ANTI-TAC DEX (CONJ CON-A TPA) 3H-TDR)  
(ADDITIVE-PARTIAL-CAUSES CON-A TPA TAC)  
(BLOCK DEX CON-A TAC)  
(CAUSE CON-A IFN)  
(NOT (CAUSE IL2 IFN))  
(PARTIAL-BLOCK DEX CON-A IFN)  
(PARTIAL-CAUSE IL2 IFN)  
(RESCUE IL2 DEX CON-A TAC)
```

(UP-REGULATE IL2 CON-A IFN)

...

This program output states the following causal conclusions:

- These results are derived from experiments on T-cells.
- The events ANTI-TAC and DEX both partially block the causation by the conjunction of the events CON-A and TPA of 3H-TDR, and these partial blocks are additive. That is, that the occurrence of both ANTI-TAC and DEX causes a greater block of the causation of 3H-TDR than either ANTI-TAC or DEX alone.
- The events CON-A and TPA both partially cause the occurrence of the event TAC, and these partial causes are additive.
- The event DEX blocks the causation of TAC by CON-A.
- The event CON-A causes the occurrence of the event IFN.
- The occurrence of the event IL2 does not cause the occurrence of the event IFN.
- DEX partially blocks the causation of IFN by CON-A.
- IL2 partially causes the event IFN.
- IL2 rescues the block by DEX of the causation of TAC by CON-A. That is, experimentally causing the two events DEX and CON-A does not cause the occurrence of the event TAC, but experimentally causing the three events IL2, DEX and CON-A does cause the occurrence of TAC.
- IL2 causes an increase in the causation of IFN by CON-A, but IL2 by itself does not cause IFN.

Finally, the user can query this database of causal model statements to locate experimental data that underlies specific causal statements.

1.5 Contributions

This thesis introduces a vocabulary of causal reasoning derived from studying experimental biologists. This vocabulary is aimed at formalizing causal language used by biologists, and at capturing causal reasoning methods used by biologists. The thesis introduces a set of causal relations between events; methods for deriving these relations from empirical data; and methods for reasoning with these relations. This theory is embodied in a program that takes as input the raw data from a biology publication and produces as output a causal model of the system under study. The motivating application for this theory is the construction of an intelligent information retrieval system that could retrieve experimental reports based on queries about causal models. This program has been empirically tested by comparing its performance to the results obtained by experienced biologists working on the same task. This thesis also presents an analysis of the space of causal relations.

This thesis differs from most previous work by developing a richer causal vocabulary; by reasoning directly from experimental data to causal models; and by empirically testing these techniques against biologists.

1.6 Thesis Organization

Chapter 2 describes the knowledge representation introduced in this thesis. Chapter 3 describes representative samples of the inference rules introduced in this thesis, and illustrates inferences performed using these rules. Chapter 4 describes empirical tests of the representation and inference rules introduced in Chapters 2 and 3. The system is tested on the task of inferring causal models from experimental data. The performance of the system is compared with that of experienced biologists performing the same task. Chapter 5 discusses some related work, and Chapter 6 presents several directions for future research.

Chapter 2

Representing Causal Relations

This chapter describes the knowledge representation introduced in this thesis.

Developing a knowledge representation involves making design decisions. There is a tension between the goal of having a minimalistic formal system and the goal of capturing the common sense ways that people reason. The representation introduced in this thesis uses a rich vocabulary of causal relations, rather than a minimal set (Table 2.1). The motivation for this decision was the desire to capture the causal language used by biologists.

There are different levels of abstraction in this representation. One division is the difference between considering events to be all-or-nothing, and considering that events can partially occur. This chapter first presents causal relations of the first type, and then presents causal relations of the second type.

2.1 Ontology

In order to focus on the causal reasoning task, this thesis uses a very simple ontology. A physical system is represented by a set of events. An experiment is performed by starting with several copies of equivalent systems, and conducting one or more experimental trials. In each trial, a set of events is externally caused, some period of time is allowed to elapse, and then a set of measurements are taken. The complete description of an experiment consists of: which events were externally caused, which measurements were performed, and the results of these measurements. Each measurement is taken to be an indicator of whether or not the event that corresponds to the measurement occurred, and the level at which it

Leads-To
Constitutive
Cause
Require
Prevent
Block
Rescue
Necessary
Associated
Involve-Nec
Involve-Suf
On-Same-Pathway
Upstream
Downstream
On-Parallel-Pathways
Cause-Only
Prevent-Only
Partial-Cause
Partial-Block
Partial-Rescue
Additive-Partial-Causes
Additive-Partial-Blocks
On-Parallel-Additive-Pathways
Up-Regulate
Down-Regulate

Table 2.1: The causal relations introduced in this thesis.

occurred.

A causal model is a set of causal statements. A causal statement consists of a causal relation and a set of event arguments. A conjunction of events can also be an argument to a causal relation. For example, the causal statement that the occurrence of both event A and event B causes event C to occur is represented as $\text{cause}(\text{conj}(A,B),C)$.

One of the central organizing principles used by biologists to think about physiological systems is the concept of a pathway. Informally, a causal pathway is a set of events, such that the occurrence of the first event causes the occurrence of the second event, which in turn causes the occurrence of the third event, etc. A causal pathway may also contain forks, and conjunctive, disjunctive and additive merges.¹ We will use this informal concept of pathway in explaining causal reasoning.²

2.2 Causal relations: all-or-nothing events

This section describes the causal relations that deal with events as all-or-nothing occurrences (i.e., an event either occurs, or it does not occur).

2.2.1 Leads-To

The leads-to causal relation is the only relation (in the all-or-nothing set) used to represent experimental observations. The system presented in this thesis does not infer leads-to relations; they are used only as input to the program.

Leads-to(A,B) means that, in some experiment, event A has been observed to occur (perhaps caused to occur), and then, within some period of time³ event B has been observed to occur.

¹Although many cellular pathways contain feedback loops, for the purposes of this thesis we will not consider feedback loops. This simplification can be supported by the fact that feedback loops were not, and did not need to be, discussed in the biology articles from which the reasoning techniques in this thesis were derived.

²The precise definition of a pathway is one of the more contentious issues in attempts to formalize biology. Different systems use different definitions of pathway, which can lead them to return differing answers to the same biological question. The representation presented in this thesis avoids the difficulty of precisely defining the concept of a pathway by not explicitly representing pathways as objects in the representation. As described above, the ontology used in this thesis consists only of events and causal relations between them. There are no pathway entities in the representation.

³The amount of time that elapses between event A and event B is not represented in this representation.

2.2.2 Constitutive

Constitutive is the only unary causal relation in this representation. The meaning of constitutive(A) is that event A normally occurs in the system under study (during the course of a standard experiment) without an external cause. (Here, as in many places, the program relies on the biologists to publish experiments that portray systems in “normal” conditions.

2.2.3 Cause

Cause is the central relation in causal modeling. Cause(A,B) means that if event A occurs, then, within some period of time, event B will occur.

The difference between cause and leads-to is that cause represents a causal relationship, whereas leads-to represents a—perhaps accidental—observed correlation. In other words, even though it is observed that leads-to(A,B), A might have nothing to do with B. For example, B might be constitutive.

2.2.4 Require

Cause(A,B) states that the occurrence of event A is sufficient to cause the occurrence of event B. A related question is whether the occurrence of event A is required for the occurrence of event B. That is, can event B occur if event A does not occur? If it cannot, then event A is required for event B. The statement that the occurrence of event A is required for the occurrence of event B is represented as require(A,B). Note that a required event may be either constitutive, or it may be externally caused.

2.2.5 Prevent

So far we have considered situations in which the occurrence of one event causes the occurrence of another event. Another important type of causal relation involves the occurrence of one event preventing the occurrence of another event. The statement that event B cannot occur after event A occurs is represented as prevent(A,B).

2.2.6 Block

$\text{Prevent}(A,B)$ states that event A prevents the occurrence of event B, no matter which causes of B might occur. Often, rather than preventing event B from occurring under all circumstances, event A might block one specific cause of B, but not block other causes of B. For example, if C causes B, and D causes B, A might block C's causation of B, but not block D's causation of B. That is, the occurrence of A and C would not lead to B, whereas the occurrence of A and D would lead to B. This situation would be represented by $\text{block}(A,C,B)$ and $\text{not}(\text{block}(A,D,B))$.

2.2.7 Rescue

An important class of biological experiments are “knockout and rescue” experiments. In these experiments, the function of an object is determined by removing (“knocking out”) the object from the system, and observing the consequences for the system. If knocking out an object causes a defect in the functioning of the system, then the experiment provides some evidence that that object is necessary for that function. A typical way to obtain additional evidence as to the role of the deleted object is to test if the defect caused by the deletion can be “rescued” by the addition of another object (perhaps one with a known function). The rescue causal relation is used to represent this type of situation. If $\text{leads-to}(A,B)$, and $\text{not}(\text{leads-to}(\text{conj}(A,C),B))$ (which entail that $\text{block}(C,A,B)$), and yet $\text{leads-to}(\text{conj}(D,A,C),B)$, then $\text{rescue}(D,C,A,B)$, which can be read as “D rescues the block by C of the pathway from A to B.”

2.2.8 Necessary

$\text{Necessary}(A,B,C)$ states that A is necessary for the pathway from B to C. That is, if A is prevented, then the pathway is blocked. $\text{Necessary}(A,B,C)$ does not determine whether A is caused by B; A might, for example, occur constitutively.

2.2.9 Associated

Biologists often describe an event as being “associated with” a pathway. In causal terms this is a relatively weak constraint, in that it implies only that the event is caused by the

initial events in the pathway. This does not guarantee that there is any situation in which the event is necessary or sufficient for the pathway. However, this type of correlation often suggests hypotheses for further verification.

Associated(A,B,C) states that the event A is associated with the pathway from event B to event C. The only consequence that this statement strictly entails is that B causes A.

From a strictly logical point of view, this might seem like a weak connection between A and the pathway from B to C. However, in some domains, “associated” also has a strong heuristic component. For example, cells usually respond quite specifically to external stimuli. Therefore, if an external stimulus is known to initiate a given causal pathway, and if it is then discovered that this stimulus also causes some internal cellular event, there is reasonable grounds to hypothesize that the internal event is somehow associated with the pathway.

For example, from the observation that stimulating a T cell with antigen causes an increase in cytoplasmic calcium, scientists hypothesized that this increase in cytoplasmic calcium might be involved in the pathway that leads from antigen stimulation to cell proliferation (which is the cell’s main response to antigen).⁴

This heuristic value of “associated” could apply in any domain where there is an underlying assumption that the systems are designed to have specific responses to external stimuli. This would seem to include nearly all evolved living systems as well as most designed artifacts.

2.2.10 Involve-Necessary

Involve-nec(A,B,C) states that A is associated with the pathway from B to C, and that it is necessary for the pathway. A is therefore a necessary intermediate event in the pathway from B to C. (See Section 3.2.)

⁴This line of reasoning also involves a certain classification of events based on their rough “causal neighborhoods.” For example, this particular inference is strengthened by the fact that antigens are external to the cell, that cell proliferation is initiated in the innermost part of the cell (the nucleus), and that an increase in cytoplasmic calcium is located in a region inside of the cell, but outside of the nucleus. Roughly, since the signal must be transmitted from outside the cell to the nucleus, there is good reason to believe that cytoplasmic events might be involved in this pathway. (See Section 6.2.4.)

2.2.11 Involve-Sufficient

Involve-suf(A,B,C) states that A is caused by B, and that A causes C. A is therefore a sufficient intermediate event in the pathway from B to C. (See Section 3.2.)

2.2.12 On-Same-Pathway

The preceding causal relations involved the relationship between an event and a causal pathway. The following causal relations discuss the relationships between two events, with respect to a causal pathway. Typically, they involve a situation where there is more than one causal pathway from an initial event to a final event, and the question under examination is whether the two events occur on the same causal pathway, or on different causal pathways.

On-same-pathway(A,B,C,D) states that A and B are on the same pathway from C to D. This entails that either A is upstream from B on this pathway (i.e. A is closer to C than B is), or it is downstream of B.

2.2.13 Upstream

Upstream(A,B,C,D) states that A is upstream of B on the pathway from C to D. That is, A is closer to the beginning of the pathway than B is. This entails that cause(A,B), and that preventing A will block the pathway from C to B, and hence from C to D.

2.2.14 Downstream

Downstream(A,B,C,D) states that A is downstream from B on the pathway from C to D. This entails that cause(B,A), and that preventing B will block the pathway from C to B.

2.2.15 On-Parallel-Pathways

There are situations where A and B are both on pathways from C to D, yet they are on different pathways. This situation is denoted by on-parallel-pathways(A,B,C,D). This entails that neither upstream(A,B,C,D) nor downstream(A,B,C,D).

2.2.16 Cause-Only

The next two causal relations are used to represent background domain knowledge.

In any experimental field in which direct manipulation of all of the components of the underlying system is infeasible, experimental techniques are used to manipulate the systems under study. These techniques are used because their effects are known. For example, in a cell biology experiment, a scientist might add TPA to a cell culture in order to activate the molecules of PKC enzyme in the cells. An important aspect of an experimental technique is that not only do we know what the manipulation will effect, but we also know what it will not effect. For example, when biologists use TPA to activate PKC, they assume that any observed effect of the TPA must be related to TPA's activation of PKC. This assumption would be represented by cause-only(TPA,PKC).

Cause-only(A, B) entails that A causes B , and that any event other than B that is also caused by A must be caused through A 's causation of B . Formally,

$$\text{cause-only}(A,B) \supset \forall(C) [(\text{cause}(A,C) \wedge B \neq C) \supset \text{cause}(B,C)].$$

2.2.17 Prevent-Only

Analogously to cause-only, we can also represent that prevent-only(A, B). This means that A prevents B , and any other effect that A has must be caused through A 's prevention of B . Formally:

$$\text{prevent-only}(A,B) \supset \forall(C) [(\text{prevent}(A,C) \wedge B \neq C) \supset \text{require}(B,C)].^5$$

2.3 Causal relations: partial effects

The causal relations in this section deal with situations where events can occur at different magnitudes. For example, an increase in cytoplasmic concentration of calcium can occur at different magnitudes.

⁵The apparent lack of parallelism between the axiom for cause-only and the axiom for prevent-only arises from the causal “negative sign” imparted by prevent. This can also be seen in the transitivity axioms for cause and prevent:

$$\begin{aligned} & [\text{cause}(A,B) \wedge \text{cause}(B,C)] \supset \text{cause}(A,C) \\ & \text{and} \\ & [\text{prevent}(A,B) \wedge \text{require}(B,C)] \supset \text{prevent}(A,C). \end{aligned}$$

2.3.1 Partial-Cause

Partial-cause(A,B) states that A causes B, to a partial degree, which means that A causes an increase in B, but the level of B caused by A is less than the maximal level of B that can be caused by the occurrence of some other events.

2.3.2 Partial-Block

Partial-block(A,B,C) states that A partially blocks the pathway from B to C, which means that although A causes a decrease in the level of C that was caused by B, A does not completely block the effect of B on C. The level of C caused by A and B is still greater than the level of C that is observed when B does not occur.

2.3.3 Partial-Rescue

Partial-rescue(A,B,C,D) states that A partially rescues the block by B of the causation by C of D, but that A does not fully restore D to the level caused by C alone.

2.3.4 Additive-Partial-Causes

Additive-partial-causes(A,B,C) states that A and B are partial causes of C, and that their effects are synergistic. A and B together cause a greater level of C than either of them causes alone.

2.3.5 Additive-Partial-Blocks

Additive-partial-blocks(A,B,C,D) states that both A and B are partial blocks of the pathway from C to D, and that their effects are additive. A and B together block the pathway from C to D more completely (i.e., result in a lower level of the occurrence of D) than either of them alone.

2.3.6 On-Parallel-Additive-Pathways

On-parallel-additive-pathways(A,B,C,D) is analogous to on-parallel-pathways, but adds the further piece of information that these pathways have synergistic effects (i.e., activating both of the pathways results in a greater level of the occurrence of D).

2.3.7 Up-Regulate

Up-regulate(A, B, C) states that A increases the partial effect that B has on C . The difference between up-regulate(A, B, C) and additive-partial-causes(A, B, C) is that up-regulate(A, B, C) implies that A alone has no effect on C .

2.3.8 Down-Regulate

Finally, down-regulate(A, B, C) states that A decreases the effect that B has on C .

2.4 An Analysis of the Space of Causal Relations

This section presents an analysis of the space of causal relations. The motivation for this analysis is the desire to provide some structure for the dozens of causal relations introduced in this chapter. This sections presents a generative system that can generate causal relations of the type used in the causal vocabulary described above. (See Figure 2-1.) This generative system consist of a set of operators. Each operator takes as input a causal relation, and produces as output a new causal relation. The following sections describe these operators.

2.4.1 Change-Effect

The starting point for the elaboration of causal relations is “constitutive”, which is the only unary causal relation. An event is constitutive if it will occur during an experiment without any external cause.

Assume that an event E is not constitutive; that is, that in a control experiment where no external interventions are caused, E will not occur.

Now assume that externally causing event C to occur results in event E also occurring. We can represent this causal relation by “cause(C, E)”. The transformation from “constitutive” to “cause” is an example of the “change-effect” operator: the addition of a new cause (C) changes the occurrence or non-occurrence of the effect E .

If we apply the change-effect operator again, to represent the causal relation where adding a new antecedent event B to the current antecedent event C now causes E not to occur, the result is the ternary causal relation “block(B, C, E)”.

If we apply the change-effect operator again, adding a new antecedent event R , the

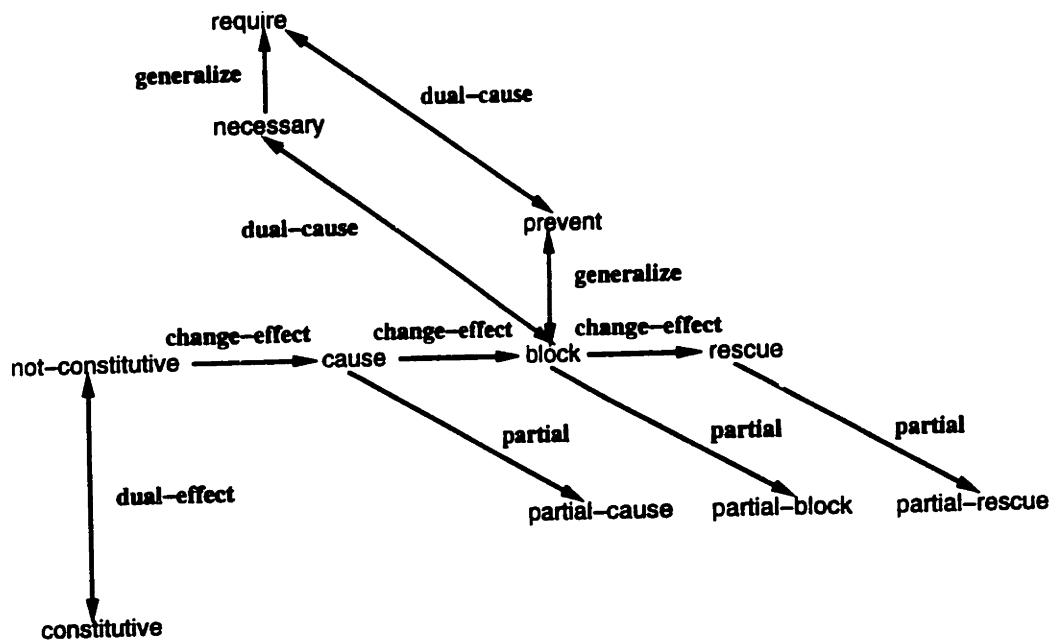


Figure 2-1: An analysis of the space of causal relations.

result is a causal relation where the conjunction of the antecedent events C, B and R now leads to the effect E occurring. This is the quaternary causal relation “rescue(R,B,C,E)”.

This application of the change-effect operator can be continued, generating a 5-ary causal relation, etc., however there is no empirical justification from the biological reasoning domain to support the construction of this causal relation.

2.4.2 Dual-Effect

The dual-effect operator transforms a given causal relation into another causal relation where the same causes lead to the opposite effect. For example, the result of applying dual-effect to “constitutive” is “not-constitutive”, the unary causal relation that states that an event will not occur without an external cause.

The result of applying dual-effect to “cause” is “extinguish”. The binary causal relation “extinguish(C,E)” that states that if C does not occur then E will occur, and if C does occur then E will not occur. Extinguish is also the result of applying the “change-effect” operator to the “constitutive” causal relation. (It appears that neither extinguish–nor any higher-arity causal relations that could be generated by continuing to apply the change-effect operator–are in common use in biological reasoning.)

2.4.3 Dual-Cause

The dual-cause operator transforms a causal relation where the occurrence of an antecedent event has some effect into another causal relation where the non-occurrence of the antecedent effect has the same effect. For example, the result of applying the dual-cause operator to the causal relation “block(B,C,E)” is the causal relation “necessary(B,C,E)”. Block(B,C,E) represents the causal model statement that the occurrence of event C will cause the occurrence of event E, and that the occurrence of event B will block this causation. Necessary(B,C,E) represents the causal relation where C causes E, but if B does not occur, then C will not cause E. That is, the occurrence of B is necessary for the causal pathway from C to E. (This relation is sometimes called “enablement” in the Artificial Intelligence literature [16].)

2.4.4 Generalize

The generalize operator transforms a n-ary causal relation into an (n-1)-ary causal relation by universally quantifying over the last of the antecedent events. For example, the result of applying the generalize operator to the causal relation $\text{block}(B,C,E)$, is the causal relation $\text{prevent}(B,E)$. $\text{Block}(B,C,E)$ represents the causal model where the occurrence of event B blocks the causation, by event C, of event E. $\text{Prevent}(B,E)$ represents the causal model where B blocks all of the causal pathways that lead to the occurrence of event E. For example, if a biologist observes that $\text{block}(B,C_1,E)$, and $\text{block}(B,C_2,E)$, etc., they might induce that $\text{prevent}(B,E)$.

2.4.5 Partial-Effect

The partial-effect operator transforms a causal relation where the occurrence of some antecedent event leads to the occurrence of some consequent event, into a causal relation where the occurrence of the antecedent event leads to the partial occurrence of the consequent event. For example, the result of applying the partial-effect operator to the cause relation is the partial-cause relation.

This chapter has introduced the knowledge representations used in this thesis. It has also presented an analysis of the space of causal relations as a way of understanding the relationships between causal relations. The next chapter presents inference methods that operate on the causal models described in this chapter.

Chapter 3

Causal Reasoning

This chapter describes six sets of inference rules for inferring causal models from experimental data. In each section, the inference rules are first presented in abstract form, and are then followed by examples from the biological literature.

The first section describes rules for inferring basic causal relationships between events, as for example, inferring from observations that one event causes another event. The second section describes rules for inferring which of the events in a causal pathway are necessary and which are sufficient. The third section describes rules for inferring the placement of events in a causal pathway, as for example, inferring which of two events occurs earlier in the pathway. The fourth section describes rules for inferring the existence of multiple causal pathways and the relationships between these pathways, as for example, inferring that there are two parallel, additive pathways leading from a specific initial event to a specific final event. The fifth section describes rules for inferring the placement of events on different causal pathways, as for example, inferring whether two events occur on the same pathway or on parallel pathways. The sixth section describes rules for inferring whether an event regulates a causal pathway.

Notation

Throughout this chapter, single italic upper case letters (A, B, C, \dots) will be used to denote event variables. The names of actual biological events will all contain more than one letter. The null event, i.e. a situation where no event is caused to occur, will be denoted by \emptyset . The

conjunction of the events A and B will be denoted by “ $\text{conj}(A, B)$.” In order to simplify the presentation of the causal inference rules, the standard predicate calculus inference rules—such as Conjunction Introduction and Conjunction Elimination—are not presented in this chapter. Similarly, although many of the rules have contrapositive and converse forms, these are only shown where necessary.

The causal relations described in Chapter 2 are listed in Table 3.1.

When describing a causal pathway, we will refer to as initial events those events that only have exogenous causes, and we will refer to as final events those whose only effects are entirely exogenous to the pathway. The remaining events in a pathway will be referred to as intermediate events.

For the simple quantity system used in this thesis, the measurement of the level of activity of event A , in the situation where event B is caused to occur, will be denoted by “ $\text{measure}(A, B)$ ”. These measurements, along with the constant 0, are the only quantity terms used in this chapter. Three comparison predicates will be used to compare these quantity terms: significantly greater than (\gg), significantly less than (\ll), and approximately equal to (\approx).

3.1 Inferring basic causal relations from observations

This section illustrates the most basic forms of causal reasoning, using three relations. If an experimenter observes that causing event A to occur results in event B subsequently occurring, then we say that $\text{leads-to}(A, B)$. Leads-to is the primitive in which all binary (all-or-nothing) observations are expressed.¹ If a controlled experiment demonstrates that $\text{leads-to}(A, B)$, and that, under the same conditions, not causing A results in B not occurring, then we write that $\text{cause}(A, B)$. If $\text{cause}(A, B)$, but the combination of A and C does not lead to B , then we write that $\text{block}(C, A, B)$, which can be read as “ C blocks the pathway from A to B ,” If we then find that the combination of A and C and D does lead to B , while D alone does not lead to B , then we say that “ D rescues the block by C of the pathway from A to B ”, which we write as $\text{rescue}(D, C, A, B)$. Although we could continue iterating these relations—perhaps some E cancels the rescue of the block—we will stop at this level,

¹In Sections 3.4 through 3.6, we will use measure to express qualitative measurements of continuous values.

Leads-To
Constitutive
Cause Require Prevent Block Rescue
Necessary Associated Involve-Nec Involve-Suf
On-Same-Pathway Upstream Downstream On-Parallel-Pathways
Cause-Only Prevent-Only
Partial-Cause Partial-Block Partial-Rescue
Additive-Partial-Causes Additive-Partial-Blocks
On-Parallel-Additive-Pathways
Up-Regulate Down-Regulate

Table 3.1: Causal relations.

Rule 3.1.1

$$\frac{\text{leads-to}(A, B) \quad \text{not}(\text{leads-to}(\emptyset, B))}{\text{cause}(A, B)}$$

Rule 3.1.2

$$\frac{\text{cause}(A, B) \quad \text{not}(\text{leads-to}(\text{conj}(A, C), B))}{\text{block}(C, A, B)}$$

Rule 3.1.2-N-2

$$\frac{\text{cause}(A, B) \quad \text{leads-to}(\text{conj}(A, C), B)}{\text{not}(\text{block}(C, A, B))}$$

Rule 3.1.3

$$\frac{\text{block}(C, A, B) \quad \text{leads-to}(\text{conj}(A, C, D), B) \quad \text{not}(\text{cause}(D, B))}{\text{rescue}(D, C, A, B)}$$

Figure 3-1: Rules for introducing causal relations. Note that we have listed one of the negative forms of one of the rules. Rule 3.1.2-N-2 is the rule formed by negating the second clause in Rule 3.1.2.

because our biological evidence provides strong motivation only up to this level. These inference rules are shown in Figure 3-1.

Biological Examples

This section presents an example from the biological literature [15] to illustrate the reasoning methods discussed above.

In these experiments, several drugs are added to a T-cell culture, and the effects on the expression of Interleukin 2 receptors (IL2R) are observed. Untreated cells do not express Interleukin 2 receptors. Injecting Concavilin A (Con A) into the cell culture leads to expression of Interleukin 2 receptors. However, injecting Concavilin A and Dexamethasone (Dex) does not lead to expression of Interleukin 2 receptors. Injecting Interleukin 2 (IL2) alone also does not lead to expression of Interleukin 2 receptor. However, injecting all three drugs: Concavilin A, Dexamethasone and Interleukin 2 does lead to expression of Interleukin 2 receptor.

1	<code>not(leads-to(∅, IL2R))</code>	observation
2	<code>leads-to(ConA, IL2R)</code>	observation
3	<code>not(leads-to(conj(ConA, Dex), IL2R))</code>	observation
4	<code>leads-to(conj(ConA, Dex, IL2), IL2R)</code>	observation
5	<code>not(leads-to(IL2, IL2R))</code>	observation
6	<code>cause(ConA, IL2R)</code>	3.1.1 (1,2)
7	<code>block(Dex, ConA, IL2R)</code>	3.1.2 (6,3)
8	<code>rescue(IL2, Dex, ConA, IL2R)</code>	3.1.3 (7,4,5)

Figure 3-2: Inferring simple causal relations (from Reem & Yeh 1985).

From these results we can infer that Con A² causes IL2R; that Dex blocks the pathway from Con A to IL2R; and that IL2 rescues the block, by Dex, of the pathway from Con A to IL2R.

These inferences are shown in Figure 3-2. These figures use a Natural-Deduction format. The left column contains a sequential line number; the middle column contains the causal statements and the right column contains the justification for the corresponding statement. In some cases the justification is an experimental observation; these lines are marked “Observation.” In other cases, the justification might be that the statement is a widely-accepted assumption about the experimental techniques in the field. The statements about these experimental techniques are often stated in terms of cause-only: i.e., a statement that a given experimental technique *A* causes an event *B*, and that *B* is the only direct effect that *A* will have on the cell.³ Analogously, a statement about an experimental technique may be phrased in terms of prevent-only: i.e., a statement that a given experimental technique *A* prevents an event *B*, and that preventing *B* is the only direct effect that *A* will have on the cell.

The other main type of justification is used when a statement is inferred by using one of the inference rules. In this case, the justification consists of two parts: the number of the applicable rule, and the line numbers of the antecedents of this inference. (For conciseness, a statement is sometimes marked as “given”. In these cases, the statement can be derived from rules presented in earlier sections.)

²For brevity, we will abbreviate the complete phrasal descriptions of cellular events using short acronyms. For example, “Con A” will be used as shorthand for “the appearance of Con A in the environment of a cell.”

³Any other event that occurs must be caused by *B*.

3.2 Determining the necessary and sufficient events on a causal pathway

This section illustrates methods for determining which events are necessary and which are sufficient for a given causal pathway. We can determine that an event is sufficient for a pathway if it is caused by the initial event in the pathway⁴, and if it in turn causes the final events in the pathway. We can determine that an event is necessary for a pathway if preventing this event from occurring blocks the pathway. Note that a necessary event (like any event) may either be constitutive (it may normally occur without an external cause), or it may be caused by the initial events in the pathway. Some inference rules for determining the relation of an event to a pathway are shown in Figure 3-3. This figure also shows rules for inferring the associated causal relation described in Chapter 2.

Biological Examples

Examples from the literature of the types of rules discussed in this section can be found grouped with the examples in Section 3.3.

3.3 Determining the ordering of events in a causal pathway

This section illustrates examples of inferences about the ordering of events in a causal pathway.

Assume that there is a causal pathway from event *A* to event *B*, and that there is a causal pathway from event *B* to event *C*. As a result, there is a causal pathway from event *A*, through event *B*, to event *C*. Now assume that there is an event *D* that is also on this same causal pathway. Consider the possible locations for event *D* on this pathway.⁵ It must either lie upstream of event *B* (i.e., on the causal pathway from event *A* to event *B*), or downstream of event *B* (i.e., on the causal pathway from event *B* to event *C*). Therefore, if we determine that event *D* does not lie on the pathway from event *A* to event *B*, then it must lie on the pathway from event *B* to event *C*. And vice versa, if we determine that

⁴This does not imply that the event is directly caused by the initial event; it may be indirectly caused by the initial event through several intermediate events.

⁵Assume that all of the events are distinct, i.e., that no two of these events are the same event.

Rule 3.2.1

$\text{cause}(A, B)$
 $\text{cause}(A, C)$
 $\text{cause}(C, B)$

$\text{involve-suf}(C, A, B)$

Rule 3.2.2

$\text{block}(C, A, B)$
 $\text{prevent-only}(C, D)$

$\text{necessary}(D, A, B)$

Rule 3.2.2-N-1

$\text{not}(\text{block}(C, A, B))$

$\text{prevent-only}(C, D)$

$\text{not}(\text{necessary}(D, A, B))$

Rule 3.2.3

$\text{cause}(A, B)$

$\text{cause}(A, C)$

$\text{associated}(C, A, B)$

Rule 3.2.4

$\text{associated}(A, B, C)$

$\text{necessary}(A, B, C)$

$\text{involve-nec}(A, B, C)$

Rule 3.2.4-N-2

$\text{not}(\text{necessary}(A, B, C))$

$\text{not}(\text{involve-nec}(A, B, C))$

Figure 3-3: Rules for inferring necessary and sufficient events for a pathway.

event D does not lie on the pathway from event B to event C , then it must lie on the pathway from event A to event B . These inference rules are shown in Figure 3-4.

Biological Examples

This section presents an example from the biological literature to illustrate these rules. This example (from [11]) concerns the role of intracellular calcium, and of Interleukin 2 (IL2), in the pathway from Phytohemagglutinin (PHA) to cell division. Adding PHA to a cell culture causes the cells to synthesize DNA (DNA), which is an indication of cell division. Adding PHA also causes an increase in intracellular calcium (Ca-incr). Preventing Ca-incr

Rule 3.3.1

involve-suf(C, A, B)
involve-nec(D, A, B)
not(involve-nec(D, A, C))
involve-nec(D, C, B)

Rule 3.3.2

involve-suf(C, A, B)
involve-nec(D, A, B)
not(involve-nec(D, C, B))
involve-nec(D, A, C)

Figure 3-4: Rules for determining the ordering of events in a pathway.

blocks the pathway from PHA to DNA. Adding PHA also causes the appearance of IL 2 in the environment of the cell (by causing the cells to secrete IL 2), and the appearance of Interleukin 2 receptors on the surface of the cell (IL2R). And adding IL 2 to a culture causes the cells to synthesize DNA. However, adding IL 2 does not cause Ca-incr.

From this evidence, we can infer that there is a pathway from PHA, through the conjunction of IL2 and IL2R, to the synthesis of DNA.⁶ We can also infer that Ca-incr is necessarily involved in the pathway from PHA to DNA. However, EGTA (which prevents Ca-incr) does not block the pathway from the conjunction of IL2 and IL2R to DNA. Therefore, Ca-incr cannot be necessarily involved in the pathway from the conjunction of IL2 and IL2R to DNA. But it must necessarily be involved somewhere in the pathway from PHA to DNA. Therefore, we can infer that Ca-incr must be necessarily involved in the pathway from PHA to the conjunction of IL2 and IL2R. The trace of this inference is shown in Figure 3-5.

⁶This inference requires some domain knowledge that is beyond the scope of this chapter. Basically, a biologist knows that any effect that Interleukin-2 has on a cell must be a result of Interleukin-2 binding to Interleukin-2 Receptor. This is because Interleukin-2 is a type of molecule that only effects cells through binding to its specific receptor. Therefore, if in some situation IL2 causes an observed event, it can be assumed that IL2R has also occurred, and that the conjunction of IL2 and IL2R caused this observed event. A later version of the program tested in Chapter 4 can encode and use this background information using a new causal relation similar to cause-only.

1	cause(PHA, DNA)	given
2	cause(PHA, conj(IL2, IL2R))	given
3	cause(conj(IL2, IL2R), DNA)	given
4	cause(PHA, Ca-incr)	given
5	block(EGTA, PHA, DNA)	given
6	not(block(EGTA, conj(IL2, IL2R), DNA))	given
7	prevent-only(EGTA, Ca-incr)	experimental technique
8	involve-suf(conj(IL2, IL2R), PHA, DNA)	3.2.1 (1,2,3)
9	necessary(Ca-incr, PHA, DNA)	3.2.2 (5,7)
10	associated(Ca-incr, PHA, DNA)	3.2.3 (1,4)
11	involve-nec(Ca-incr, PHA, DNA)	3.2.4 (10,9)
12	not(necessary(Ca-incr, conj(IL2, IL2R), DNA))	3.2.2-N-1 (6,7)
13	not(involve-nec(Ca-incr, conj(IL2, IL2R), DNA))	3.2.4-N-2 (-,12)
14	involve-nec(Ca-incr, PHA, conj(IL2, IL2R))	3.2.5 (8,11,13)

Figure 3-5: Determining the ordering of events on a pathway (from Mills et al. 1985).

3.4 Determining the relationship between two parallel pathways

This section illustrates examples of inferring the existence of two parallel pathways. To do this, it will be necessary to consider the magnitudes of events (e.g., the level of DNA synthesis observed).

Rule 3.1.2 in Section 3.1 described an inference based on the complete block of an effect. Consider what can be inferred by a partial block of an effect. Assume that the occurrence of event *A* leads to event *B* occurring at a certain level (call it 100%). Now assume that the occurrence of events *A* and *C* leads to event *B* occurring at a 50% level. We can say that *C* partially blocks the pathway from *A* to *B*. If we assume that *C* achieves its effect by completely⁷ preventing some event *D*, then we can conclude that preventing *D* partially blocks the pathway from *A* to *B*.

If we further assume that preventing *D* achieves its effect by completely blocking the pathway on which *D* occurs, then we are forced to conclude that there is another pathway, that runs from *A* to *B*, and that does not involve *D*. We can, without loss of generality, name some event on this other pathway using the event variable *E*, and we can then state

⁷This assumption is based on the experimental practice of using increasing drug dosages until we reach a point where a greater concentration of the drug does not elicit any greater magnitude of the response. We can therefore assume that whatever event the drug is preventing has been completely suppressed.

Rule 3.4.1

$\text{measure}(C, \text{conj}(A, B)) \ll \text{measure}(C, B)$

$\text{measure}(C, \text{conj}(A, B)) \gg 0$

$\text{partial-block}(A, B, C)$

Rule 3.4.2

$\text{partial-block}(A, B, C)$

$\text{prevent-only}(A, D)$

$\text{on-parallel-additive-pathways}(D, E, B, C)$

Figure 3-6: Rules for inferring parallel additive pathways. Note that E in the conclusion of Rule 3.4.2 is an anonymous variable. This implies that we know that there is a pathway through some event other than D , but we don't know the identity of that event.

that D and E are on parallel pathways from A to B . Furthermore, we know that these are parallel, additive pathways. These inference rules are shown in Figure 3-6.

Biological Examples

An example of this type of reasoning is found in [12]. These experiments are concerned with elucidating the pathway through which Platelet-Derived Growth Factor (PDGF) causes cell division (as indicated by DNA synthesis). The experimenters used Pertusis-Toxin (PTX) to probe this pathway, since it is known that PTX prevents the activation of a certain Guanidine nucleotide binding protein (G-protein).

The experimenters found that PTX partially blocked the pathway from PDGF to DNA. From this they concluded that there must be parallel, additive pathways from PDGF to DNA, and that G-protein must lie on one of these pathways. These inferences (along with the examples for Section 3.5) are shown in Figure 3-8.

3.5 Determining whether two events occur on the same pathway

This section illustrates examples of inferring whether two events occur on the same pathway, or on different pathways. Let us continue the example in the previous section. There we inferred, from the fact that C partially blocked the pathway from A to B , that there were multiple, additive pathways from A to B ; that C completely blocked one of these pathways;

Rule 3.5.1

partial-block(A, B, C)
partial-block(D, B, C)
 $\text{measure}(C, \text{conj}(A, B)) \approx \text{measure}(C, \text{conj}(A, B, D))$
 $\text{measure}(C, \text{conj}(D, B)) \approx \text{measure}(C, \text{conj}(A, B, D))$
not(additive-partial-blocks(A, D, B, C))

Rule 3.5.2

not(additive-partial-blocks(A, D, B, C))
prevents-only(A, E)
prevents-only(D, F)
on-same-pathway(E, F, B, C)

Figure 3-7: Rules for inferring that two events are on the same pathway.

and that C did so by preventing the occurrence of some event D , that is on one of these pathways.

Now, assume that we obtain the same results for a different event F . That is, that F partially blocks the pathway from A to B . And assume that we go through the same reasoning steps as above, and conclude that F must prevent some event F that is on some pathway that runs from A to B .

The question now arises as to whether D and F are on the same pathway, or on different pathways. One way to determine this is to try an experiment where both A, C and F are caused to occur, and to observe the resulting magnitude of B . If the observed magnitude of B in this situation is the same as the magnitude of B in the situations where either C or F occur alone (along with A), then we can conclude that the effects of C and F (in blocking the pathway from A to B) are not additive. From this we can infer that D and F occur on the same pathway from A to B , and not on parallel pathways.⁸ These inference rules are shown in Figure 3-7.

⁸In this, as in most of the examples in this thesis, there are other admissible models that would fit the observations. However, the aim in this thesis has been to capture the types of reasoning that biologists use. Biologists employ a version of Occam's Razor, and prefer the simplest model (according to their implicit metrics). We have endeavored to capture these preferences in the reasoning strategies in this thesis.

1	$\text{measure}(\text{DNA}, \text{conj}(\text{PDGF}, \text{PTX}))$	
	$\ll \text{measure}(\text{DNA}, \text{conj}(\text{PDGF}))$	observation
2	$\text{measure}(\text{DNA}, \text{conj}(\text{PDGF}, \text{PTX})) \gg 0$	observation
3	$\text{measure}(\text{DNA}, \text{conj}(\text{PDGF}, \text{pre-TPA}))$	
	$\ll \text{measure}(\text{DNA}, \text{conj}(\text{PDGF}))$	observation
4	$\text{measure}(\text{DNA}, \text{conj}(\text{PDGF}, \text{pre-TPA})) \gg 0$	observation
5	$\text{measure}(\text{DNA}, \text{conj}(\text{PDGF}, \text{PTX}))$	
	$\approx \text{measure}(\text{DNA}, \text{conj}(\text{PDGF}, \text{PTX}, \text{pre-TPA}))$	observation
6	$\text{measure}(\text{DNA}, \text{conj}(\text{PDGF}, \text{pre-TPA}))$	
	$\approx \text{measure}(\text{DNA}, \text{conj}(\text{PDGF}, \text{PTX}, \text{pre-TPA}))$	observation
7	$\text{prevent-only}(\text{PTX}, \text{G-protein})$	exper. technique
8	$\text{prevent-only}(\text{pre-TPA}, \text{PKC})$	exper. technique
9	$\text{partial-block}(\text{PTX}, \text{PDGF}, \text{DNA})$	3.4.1 (1,2)
10	$\text{partial-block}(\text{pre-TPA}, \text{PDGF}, \text{DNA})$	3.4.1 (3,4)
11	$\text{on-parallel-additive-pathways}(\text{G-protein}, E, \text{PDGF}, \text{DNA})$	3.4.2 (9,7)
12	$\text{on-parallel-additive-pathways}(\text{PKC}, E, \text{PDGF}, \text{DNA})$	3.4.2 (10,8)
13	$\text{non-additive-partial-block}(\text{PTX}, \text{pre-TPA}, \text{PDGF}, \text{DNA})$	3.5.1 (9,10,5,6)
14	$\text{on-same-pathway}(\text{G-protein}, \text{PKC}, \text{PDGF}, \text{DNA})$	3.5.2 (13,7,8)

Figure 3-8: Reasoning about parallel pathways.

Biological Examples

We will continue the biological example from Section 3.4. In that section, we had seen how Nishizawa et al. had inferred, from the partial block by PTX of the pathway from PDGF to DNA, that G-protein must lie on one of a set of parallel, additive pathways from PDGF to DNA.

An analogous line of reasoning leads to the conclusion that PKC, whose activation is prevented by pretreatment with TPA (pre-TPA), also lies on one of a set of parallel, additive pathways from PDGF to DNA. Finally, the observation that these two partial blocks are not additive leads to the conclusion that the two events (G-protein and PKC) that are prevented by these two experimental techniques (PTX and pre-TPA) must lie on the same pathway. Since this pathway is totally blocked by either of these techniques, using them together has no additional effect compared with using each alone. These inferences are shown in Figure 3-8.

Rule 3.6.1

$\text{measure}(A, B) \gg 0$

$\text{measure}(A, \text{conj}(B, C)) \gg \text{measure}(A, B)$

$\text{measure}(A, C) \approx \text{measure}(A, \emptyset)$

up-regulate(C, B, A)

Rule 3.6.2

$\text{measure}(A, B) \gg 0$

$\text{measure}(A, \text{conj}(B, C)) \ll \text{measure}(A, B)$

down-regulate(C, B, A)

Figure 3-9: Rules for inferring regulation.

- | | | |
|---|--|---------------|
| 1 | $\text{measure}(\text{IL2R}, \text{ConA}) \gg 0$ | given |
| 2 | $\text{measure}(\text{IL2R}, \text{conj}(\text{ConA}, \text{IL2})) \gg \text{measure}(\text{IL2R}, \text{ConA})$ | given |
| 3 | $\text{measure}(\text{IL2R}, \text{IL2}) \approx \text{measure}(\text{IL2R}, \emptyset)$ | given |
| 4 | up-regulate(IL2, ConA, IL2R) | 3.6.1 (1,2,3) |

Figure 3-10: Inferring regulation.

3.6 Inferring regulation

This section describes a method for inferring that an event regulates a pathway, i.e. it changes the level of the final event in the pathway. If event A causes event B to occur at a certain level, and the conjunction of events A and C cause B to occur at a higher level, then C up-regulates the pathway from A to B . Similarly, if the conjunction of events A and C cause B to occur at a lower level than the level caused by A alone, then C down-regulates the pathway from A to B . (See Figure 3-9.)

Biological Example

An example from the biological literature of the type of inference described in this section can be found in [15], which observed that adding ConA and IL2 to cells causes a greater level of IL2R expression than adding ConA alone. (See Figure 3-10.) Note that the result here, “up-regulate(IL2, ConA, IL2R)”, is consistent with the result in Section 3.1, “rescue($\text{IL2, Dex, ConA, IL2R}$)”. In addition, the two results each convey additional information: The result here indicates that although ConA alone can cause IL2R, the level of IL2R is greater if IL2 is also added. The result in Section 3.1 indicates that although ConA alone can cause IL2R, that pathway can be blocked by Dex, and IL2 can rescue that block.

This chapter presented a selection of techniques for reasoning about causal models. The next chapter will present an empirical test of this causal reasoning.

Chapter 4

Results

This chapter presents results from a test of reasoning from experiments to causal models in molecular cell biology. The task set for the program is to accept as input the raw data from a biology publication (see Table 4.1), and to generate as output a set of causal statements about the biological system under study (see Figure 4-2). The program's causal model is then compared with the results of the same task as performed by experienced biologists (see Figures 4-5 and 4-8, and Table 4.2). Figure 4-1 shows a flowchart of the setup for the experimental test.

4.1 Materials and Methods

For this experiment, a set of three publications were selected [15, 21, 11]. These publications deal with the role of Interleukin-2, and its receptor, in the process of T-cell activation. The articles chosen appeared in either the journal *Nature*, or in *The Journal of Immunology*, during the period from January to April 1985. These articles are from three different laboratories.

The input to the program is the quantitative data taken from the figures and the tables in the articles. In general, understanding a scientific experiment entails understanding: what system was investigated (the “Materials”); what experimental manipulations were performed (the “Methods”); and what data was measured or observed (the “Results”). Experiments in the domain of modern molecular cell biology often follow a stereotypical format that can simplify the description of these three characteristics.

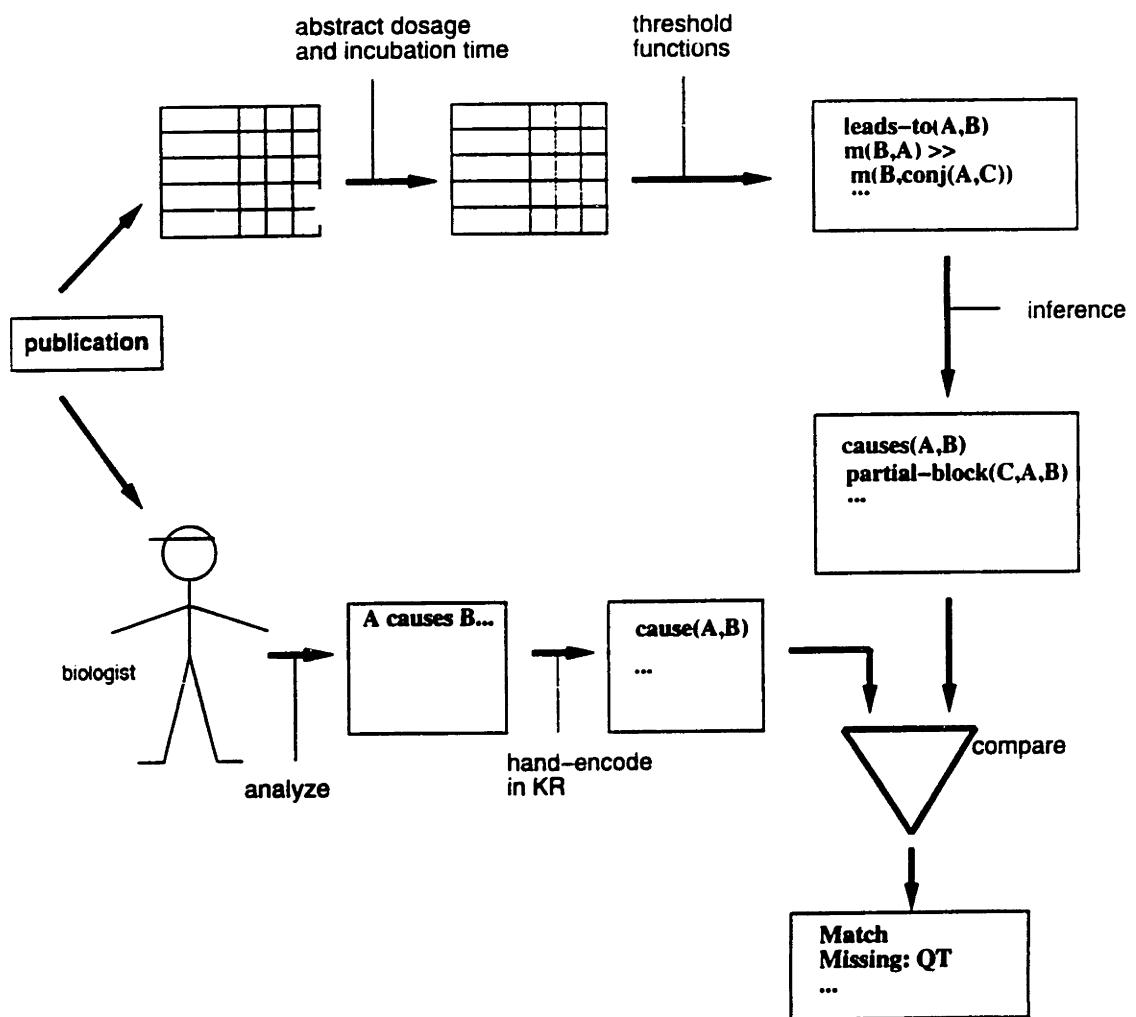


Figure 4-1: Setup for experimental test of reasoning from experiments to causal models.

additions	TAC	3H-TDR	IFN
—	1	1.0	0.0
CON-A	5	11.0	0.8
IL2	1	—	0.0
CON-A + IL2	56	300.7	6.0
CON-A + DEX	1	0.2	0.2
CON-A + IL2 + DEX	26	88.8	6.3
CON-A + IL2 + ANTI-TAC	20	73.9	2.9
CON-A + ANTI-TAC	6	9.6	0.9

Table 4.1: Reem Table 1

For example, all of the experiments discussed in this thesis are conducted by growing cells in a petri dish (or similar glass container); exposing the cells to various substances (such as hormones, ions and toxins)¹; incubating the cells for various periods (that typically range from a few hours to a few days); and then performing certain assays on the cell culture. This general format probably encompasses millions of the published biology experiments.

To a first approximation, an experiment of this type can be described by stating: what type of cells were used; which substances were the cells exposed to (and at what concentrations, and for what periods of time); and which assays were performed. At a more detailed level, it can be important to understand the exact sequence and timing of the various experimental steps. The results in this chapter demonstrate that a substantial body of conclusions can be generated by analyzing experiments represented at this simple level.

For the purposes of the causal reasoning discussed in this chapter, we shall further simplify the experiments and abstract away the details of cell-type², concentrations of stimulants and blockers, and incubation times. As a result, an experiment can be characterized by which stimulants and blockers were added to the cells, and which assays were performed. Since we are not concerned here with reasoning across different concentrations and time periods, we select those concentrations and time periods that yield the most significant results (i.e. we select the concentration and incubation time that has the greatest range between maximum and minimum measurements). An example of the data input to the program is shown in Table 4.1.

In order to convert this quantitative data into qualitative data for use by the causal

¹These substances can be functionally classified as stimulators, blockers, modulators, etc.

²All of the cells discussed in these experiments are of the same basic type: helper T-cells.

reasoning system, the program applies a simple threshold rule, loosely derived from biology lore. A difference in measurements is deemed to be significant if the two values differ by a factor of two.

The program only compares quantities within a single column (within a single table). This simple procedure allows the program to safely abstract away the experimental details enumerated above. It is the responsibility of the authors of a biology publication to ensure that they are not comparing apples and oranges (at least within a given dataset).

4.2 Results

The program was run on 15 tables extracted from the three articles in this study. One table corresponds to each dataset (figure or graph) in the articles. The complete datasets are shown in the Appendix. The results of running the program on these three articles are shown in Figures 4-2 to 4-4. These results have been abbreviated for purposes of presentation. The analyses presented in Tables 4.2 to 4.4 were done using the full output, and the full output was examined to check that the program was not producing erroneous conclusions.

A complete run of these 15 tables requires approximately 3 CPU-hours on a Sun 4/25 (with 28 MB RAM and 180 MB swap) running CMU Common Lisp 17f under SunOS 4.1.4. The vast majority (90+ %) of the time is consumed by a single experiment in the Reem article. This is due to the fact that the program considers all possible subsets of causes, which increases exponentially with the number of causitive agents. This table in the Reem article contains up to five causitive agents (Con-A, TPA, IL2, Anti-Tac, and Dex), and therefore takes significantly longer than any of the other experiments. This is an unusually high number of chemical additives for an experiment of this type.

These articles were given to experienced biologists, who were asked to prepare summaries of the conclusions of the articles. Examples of these summaries are shown in Figures 4-5 to 4-7. Encodings of these summaries in the knowledge representation were prepared by the author, and are shown in Figures 4-8 to 4-10. Tables 4.2 to 4.4 present comparisons of the program output and the biologist's results.

The representation used in the experiments presented in this chapter differs slightly from

```

(IN-CELL-TYPE T-CELL)

(ADDITIONAL-PARTIAL-BLOCKS ANTI-TAC DEX (CONJ CON-A TPA) 3H-TDR)
(ADDITIONAL-PARTIAL-BLOCKS ANTI-TAC DEX (CONJ CON-A TPA) IFN)
(ADDITIONAL-PARTIAL-CAUSES CON-A IL2 IFN)
(ADDITIONAL-PARTIAL-CAUSES CON-A TPA TAC)
(BLOCK DEX CON-A TAC)
...
(CAUSE CON-A IFN)
(CAUSE CON-A TAC)
(CAUSE TPA TAC)
(DECR (CONJ CON-A DEX) 3H-TDR)
(DECR IL2 IFN)
...
(INCR CON-A 3H-TDR)
(INCR CON-A IFN)
(INCR CON-A TAC)
(INCR IL2 IFN)
(INCR TPA TAC)
(NOT (CAUSE IL2 IFN))
(NOT (CAUSE IL2 TAC))
(NOT (CAUSE NIL IFN))
(NOT (CAUSE NIL TAC))
(NOT (CAUSE TPA IFN))
...
(PARTIAL-BLOCK ANTI-TAC (CONJ CON-A IL2) 3H-TDR)
(PARTIAL-BLOCK CSA (CONJ CON-A TPA IL2) TAC)
(PARTIAL-BLOCK DEX CON-A IFN)
...
(PARTIAL-CAUSE CON-A 3H-TDR)
(PARTIAL-CAUSE IL2 IFN)
...
(REScue IL2 (CONJ CON-A CSA) TPA TAC)
(REScue IL2 (CONJ CSA TPA) CON-A TAC)
(REScue IL2 DEX CON-A TAC)
(UP-REGULATE IL2 CON-A IFN)
(UP-REGULATE IL2 CON-A TAC)
(UP-REGULATE IL2 TPA TAC)
(UP-REGULATE TPA (CONJ CON-A IL2) IFN)
(UP-REGULATE TPA CON-A IFN)
...

```

Figure 4-2: Results from running Reem&Yeh data (abbreviated).

(IN-CELL-TYPE T-CELL)

(ADDITIONAL-PARTIAL-CAUSES A23187 TPA 3H-TDR)
(ADDITIONAL-PARTIAL-CAUSES CON-A TPA 3H-TDR)
(ADDITIONAL-PARTIAL-CAUSES IONO TPA 3H-TDR)
(BLOCK EGTA TPA 3H-TDR)
(CAUSE CON-A 3H-TDR)
(CAUSE IL2 3H-TDR)
(CAUSE TPA 3H-TDR)
(DECR (CONJ CON-A TPA) IL2R)
(DECR A23187 3H-TDR)
(DECR IONO 3H-TDR)
(DECR TPA 3H-TDR)
(INCR (CONJ IONO TPA) 3H-TDR)
(INCR (CONJ IONO TPA) IL2R)
(INCR A23187 3H-TDR)
(INCR CON-A IL2R)
(INCR IONO 3H-TDR)
(NECESSARY CA TPA 3H-TDR)
(NOT (CAUSE (CONJ IONO TPA EGTA) 3H-TDR))
(NOT (CAUSE (CONJ TPA EGTA CACL2) 3H-TDR))
(NOT (CAUSE (CONJ TPA EGTA) 3H-TDR))
(NOT (CAUSE A23187 3H-TDR))
(NOT (CAUSE IONO 3H-TDR))
(NOT (CAUSE NIL 3H-TDR))
(NOT (CAUSE TPA 3H-TDR))
(PARTIAL-BLOCK CON-A TPA IL2R)
(PARTIAL-BLOCK TPA CON-A IL2R)
(PARTIAL-CAUSE (CONJ CON-A TPA) 3H-TDR)
(PARTIAL-CAUSE (CONJ IONO TPA) 3H-TDR)
(PARTIAL-CAUSE A23187 3H-TDR)
(PARTIAL-CAUSE IONO 3H-TDR)
(PARTIAL-CAUSE TPA 3H-TDR)
(RESCUE IONO (CONJ CACL2 EGTA) TPA 3H-TDR)
(UP-REGULATE A23187 TPA 3H-TDR)
(UP-REGULATE IONO TPA 3H-TDR)
(UP-REGULATE TPA CON-A 3H-TDR)
...

Figure 4-3: Results from running Truneh et al. data (abbreviated).

(IN-CELL-TYPE T-CELL)

```
(DECR EGTA 3H-TDR)
(INCR (CONJ IL2 EGTA) 3H-TDR)
(INCR CON-A CA)
(INCR IL2 3H-TDR)
(INCR PHA CA)
(NOT (CAUSE EGTA 3H-TDR))
(ON-PARALLEL-ADDITIONAL-PATHWAYS CA ?E IL2 3H-TDR)
(PARTIAL-BLOCK EGTA IL2 3H-TDR)
(PARTIAL-CAUSE (CONJ IL2 EGTA) 3H-TDR)
...
...
```

Figure 4-4: Results from running Mills et al. data. (abbreviated).

1. IL-2 is required for expression of Tac Ag, proliferation of human thymocytes and g-IFN synthesis. Thymocytes stimulated with IL-2 alone are not induced to express IL-2 receptor, to proliferate or to synthesize interferon; however, IL-2 increases each of these effects in cells treated with ConA. Thus, IL-2 modulates expression of its receptor.
2. ConA alone somewhat stimulates expression of Tac antigen in T lymphocytes. This effect is inhibited by dexamethasone and anti-Tac antibody. Inhibition by dex can be overcome by IL-2. This is due to the fact that dex blocks IL-2 synthesis.
3. Synthesis of g-interferon and expression of Tac antigen can not be induced by TPA alone. However, TPA enhances the stimulation of interferon and Tac by ConA.
4. IL-2 can not overcome inhibition of proliferation by anti-Tac at 10^{-3} because all receptors are blocked. Treatment of thymocytes with anti-Tac can also inhibit interferon synthesis.

Figure 4-5: A biologist's conclusions for Reem and Yeh

the representation described in Chapter 2. The causal relation *Incr(A,B)* used here states that A causes an increase in the level of B. *Decr(A,B)* states that A causes a decrease in the level of B. Although the system has evolved from the time of this experiment (it can now generate some of the conclusions that it missed in this experiment), in the interests of good science, these experiments are presented in the form in which it was originally conducted. Although these early experiments demonstrate only modest success, they do provide some encouragement, some discipline, and directions for further work.

1. Calcium ionophores such as ionomycin and A23187 do not by themselves induce (cause) proliferation in mouse T lymphocytes. However, treatment of these cells with both calcium ionophore and TPA causes ^{3}H - thymidine incorporation. ConA and TPA together induce proliferation after 3 hours incubation, but proliferation is blocked after 20 or 40 hours of incubation.
2. Ionomycin and TPA-induced proliferation can be blocked by 2 mM EGTA. This effect can be reversed by addition of 2 mM CaCl_2 , but not by addition of MgCl_2 , indicating that proliferation requires calcium and the effect is calcium-specific.
3. Ionomycin and TPA together induce IL-2 secretion, but ionomycin alone cannot induce this effect.
4. ConA is more effective at inducing IL-2 secretion in the presence of TPA, but only in the 20% supernatant, and only at 48 and 72 hours.
5. IL-2 is required for cellular proliferation, as addition of neither ionomycin nor ionomycin + TPA to CTLL cells (which require exogenous IL-2 for growth) can induce proliferation. Addition of IL-2 to these cells markedly stimulates proliferation.
6. Treatment of T lymphocytes with ionomycin and TPA induces expression of the IL-2 receptor, whereas addition of ionomycin or TPA alone does not. ConA alone also causes expression of the receptor, but this expression is blocked by ConA and TPA in these cells.

Figure 4-6: A biologist's conclusions for Truneh et. al

1. Binding of IL-2 to its receptor does not cause an increase in free cytosolic calcium. In contrast, mitogenic lectins such as PHA cause a rapid (within the first 10 minutes) increase in cytosolic calcium.
2. Binding of IL-2 to its receptor is necessary for cellular proliferation. Proliferation of peripheral blood lymphocytes, which occurs following IL-2 binding, is not associated with, nor does it require, increased intracellular calcium. Mitogenic lectins, which increase cellular calcium, do not induce cellular proliferation.
3. These conclusions hold true for both murine and human cell lines expressing the IL-2 receptor (TAC). Primary human lymphocytes also show no increase in intracellular calcium following IL-2 stimulation, however, this may be due to the fact that less than 5% of these cells express the IL-2 receptor.
4. ^{3}H -thymidine incorporation into PBL cell lines is not blocked by addition of 10 mM EGTA, further supporting the notion that calcium is not required for this process.

Figure 4-7: A biologist's conclusions for Mills et al.

```
(in-cell-type t-cell)

(require il2 il2r) ;1.1
(require il2 prolific) ;1.2
(require il2 ifn) ;1.3
(not (cause il2 il2r)) ;1.4
(not (cause il2 prolific)) ;1.5
(not (cause il2 ifn)) ;1.6
(up-regulate il2 con-a il2r) ;1.7
(up-regulate il2 con-a prolific) ;1.8
(up-regulate il2 con-a ifn) ;1.9
(modulate il2 il2r) ;1.10

(partial-cause con-a il2r) ;2.1
(block dex con-a il2r) ;2.2
(block anti-tac con-a il2r) ;2.3
(rescue il2 dex con-a il2r) ;2.4

(not (cause tpa ifn)) ;3.1
(not (cause tpa il2r)) ;3.2
(up-regulate tpa con-a ifn) ;3.3
(up-regulate tpa con-a il2r) ;3.4

(not (rescue il2 anti-tac tpa prolific)) ;4.1
(block anti-tac (conj tpa con-a) ifn) ;4.2
```

Figure 4-8: Encoding of the conclusions of a biologist for Reem & Yeh

```

(in-cell-type t-cell)

(not (cause iono prolific)) ;1.1
(not (cause a23187 prolific)) ;1.2
(cause (conj iono tpa) prolific) ;1.3
(cause (conj a23187 tpa) prolific) ;1.4
(cause (conj con-a tpa) prolific) ;1.5

(block egta (conj iono tpa) prolific) ;2.1
(rescue acl12 egta (conj iono tpa) prolific) ;2.2
(not (rescue mgcl2 egta (conj iono tpa) prolific)) ;2.3
(require incr-ca prolific) ;2.4

(cause (conj iono tpa) il2) ;3.1
(not (cause iono il2)) ;3.2

(up-regulate tpa con-a il2) ;4.1

(require il2 prolific) ;5.1
(with-cell-type ctl-1 (not (cause iono prolific))) ;5.2
(with-cell-type ctl-1 (not (cause (conj iono tpa) prolific))) ;5.3
(with-cell-type ctl-1 (cause il2 prolific)) ;5.4

(cause (conj iono tpa) il2r) ;6.1
(not (cause iono il2r)) ;6.2
(not (cause tpa il2r)) ;6.3
(cause con-a il2r) ;6.4
(block tpa con-a il2r) ;6.5

```

Figure 4-9: Encoding of the conclusions of a biologist for Truneh et al.

```

(in-cell-type t-cell)

(not (cause il2-il2r incr-ca)) ;1.1
(cause pha incr-ca) ;1.2
(require il2-il2r prolif) ;1.3

(cause il2-il2r prolif) ;2.1
(not (necessary incr-ca il2-il2r prolif)) ;2.2
(not (associated incr-ca il2-il2r prolif)) ;2.3
(cause pha incr-ca) ;2.4
(not (cause pha prolif)) ;2.5

;;; 3. [This section contains conclusions about experiments using
;;; T-cells from different organisms and different organs, and is
;;; beyond the scope of this work.]

(not (block egta il2 3h-tdr)) ;4.1
(not (necessary incr-ca il2 3h-tdr)) ;4.2

```

Figure 4-10: Encoding of the conclusions of a biologist for Mills et al.

Conclusion	Status	Analysis
1.1	Missing	MB - Missing Background Knowledge
1.2	Missing	MB - Missing Background Knowledge
1.3	Missing	MB - Missing Background Knowledge
1.4	Match	
1.5	Missing	MD - Missing Data
1.6	Match	
1.7	Match	
1.8	Missing	MD - Missing Data
1.9	Match	
1.10	Match	
2.1	Match	
2.2	Match	
2.3	Contradiction	AR - Ambiguous Result
2.4	Match	
3.1	Match	
3.2	Contradiction	QT - Qualitative Threshold (3 vs. 1)
3.3	Match	
3.4	Missing	CQ - Consequence of 3.2
4.1	Missing	NC - unimplemented Negative Conclusion
4.2	OK	PD - Partial data (PARTIAL-BLOCK vs. BLOCK)

Table 4.2: Comparison of the program output and the biologist's results for Reem&Yeh (cf. Figure 4-8 and Figure 4-2).

Conclusion	Status	Analysis
1.1	Match	
1.2	Match	
1.3	OK	PD - Partial data (INCR vs. CAUSE)
1.4	OK	PD - Partial data (INCR vs. CAUSE)
1.5	OK	PD - Partial data (INCR vs. CAUSE)
2.1	Missing	MC - Missing control
2.2	Missing	CQ - Consequence of 2.1
2.3	Missing	CQ - Consequence of 2.1 and 2.2
2.4	Missing	CQ - Consequence of 2.1, 2.2 and 2.3
3.1	OK	PD - Partial data (INCR vs. CAUSE)
3.2	Missing	(see text)
4.1	Missing	(see text)
5.1	Missing	MB - Missing Background Knowledge
5.2	Missing	CT - Cell type data
5.3	Missing	CT - Cell type data
5.4	Missing	CT - Cell type data
6.1	OK	PD - Partial data (INCR vs. CAUSE)
6.2	Missing	MB - Missing Background Knowledge
6.3	Missing	MB - Missing Background Knowledge
6.4	OK	PD - Partial data (INCR vs. CAUSE)
6.5	OK	PD - Partial data (PARTIAL-BLOCK vs. BLOCK)

Table 4.3: Comparison of the program output and the biologist's results for Truneh et al. (cf. Figure 4-9 and Figure 4-3).

Conclusion	Status	Analysis
1.1	Missing	MB - Missing Background Knowledge
1.2	OK	PD - Partial data (INCR vs. CAUSE)
1.3	Missing	MB - Missing Background Knowledge
2.1	Missing	MB - Missing Background Knowledge
2.2	Missing	MB - Missing Background Knowledge
2.3	Missing	NC - unimplemented Negative Conclusion
2.4	OK	PD - Partial data (INCR vs. CAUSE)
2.5	Contradiction	AR - Ambiguous Result
4.1	OK	PD - Partial data (PARTIAL-BLOCK vs. BLOCK)
4.2	Missing	CQ - Consequence of 4.1

Table 4.4: Comparison of the program output and the biologist's results for Mills et al. (cf. Figure 4-10 and Figure 4-4).

4.3 Discussion

The performance of the program was examined by analyzing the discrepancies between the program's conclusions and the biologists's conclusions. For each of the biologist's conclusions, the status of the program was scored as one of the following:

- Match – if the program generated a conclusion that was an exact match to the biologists's conclusion;
- OK – if the program generated a conclusion that was substantively the same as the biologist's conclusion (all of these cases are “Partial Data” discrepancies, as described below);
- Missing – if the program did not generate a conclusion that matched the biologists; and
- Contradiction – if the program generated a conclusion that directly contradicted the biologist's conclusion.

In the cases where the program did not match the biologist, an analysis was performed to determine the source of the discrepancy. The discrepancies were classified into the following categories:

- Missing Background Knowledge (MB) – These are cases where the biologist's conclusion is based on background biology knowledge that is unavailable to the program.

In these cases, a causal reasoner that does not have knowledge of the domain cannot infer the conclusion from the data.

- Missing Data (MD) – These are cases where the conclusion is based on data that is unavailable to the program. For example, this data might have been lost during the process of abstracting away time and dosage information.
- Missing Control (MC) – These are cases where the program did not infer a conclusion that the biologist inferred because the data for a control experiment that the program would require for the inference was not present. For example, the program will not infer $\text{cause}(A,B)$ from $\text{leads-to}(A,B)$ unless $\text{not}(\text{leads-to}(\emptyset,B))$ is also observed. The biologist might not have felt it necessary to see this control because of background knowledge that the biologist possessed. However, several biologists have remarked that a program that spotted these instances would be a very useful tool.
- Qualitative Threshold (QT) – These are cases where the program's simple qualitative threshold led to an incorrect interpretation of the data. In the one such case in this chapter, the program inferred that $3 \gg 1$, in a column where the maximum value was 54 and the minimum value was 1.
- Partial Data (PD) – These are cases where, for example, the program concluded $\text{partial-cause}(A,B)$ and the biologist concluded $\text{cause}(A,B)$. These cases might be due to differences between the biologist and the program in evaluating quantitative data.
- Unimplemented Negative Conclusion (NC) – These are cases where the program did not infer a negative conclusion that the biologist inferred. As a way to limit the output, this system only inferred a subset of negative conclusions that might have been inferred from the data. (See Section 6.2.2.)
- Cell Type (CT) – These cases involve comparing experimental results among different types of cells. These conclusions are beyond the scope of this chapter.
- Consequence (CQ) – These are cases where a discrepancy in one conclusion is a direct consequence of a discrepancy in another conclusion upon which the first conclusion depends.

As stated above, although these early experiments demonstrate only modest success, they do provide some encouragement, some discipline, and directions for further work. Further experiments are currently in progress.

Chapter 5

Related Work

This section describes other research related to this thesis. The main areas of interest are: vocabularies of causal relations; reasoning from experimental observations to causal models; and models of the causal vocabularies and causal reasoning used in experimental biology. Rather than attempting a broad survey, the goal of this chapter is to locate the research in this thesis with respect to the closest related work.

5.1 Causal Vocabularies

Rieger made one of the earliest attempts to invent a vocabulary for representing causal mechanisms. Rieger and Grinberg [16] introduce a vocabulary of ten causal connectives (continuous and one-shot causal, continuous and one-shot enablement, continuous and one-shot state coupling, state equivalence, state antagonism, rate confluence and threshold). They use this vocabulary to construct complicated causal models of physical systems. For example, they present a model of a home heating system. Their vocabulary also includes connectives related to intentionality, optimization, and other issues.

Unfortunately, Rieger's work fell into disfavor for many years. Weld and deKleer present a clear statement of the criticism of Rieger's work:

Causal reasoning raises two central issues: (1) The vocabulary of causal primitives, and (2) the construction of device descriptions in terms of these primitives. Rieger and Grinberg focus exclusively on the first, while the rest of the papers in this chapter focus on the second. Not surprisingly, therefore, Rieger and Grin-

berg have the most elaborate vocabulary of causality of all the papers in this chapter.

The goal of their research was to study how humans might represent cause-effect knowledge about mechanisms. They present an ontology for causality with primitives such as “tendency” and “enablement”. Once a device is modeled as a cause-effect diagram, it can be simulated to produce causal explanations. Although the causal explanations that Rieger and Grinberg construct are intuitively appealing, this appeal is misleading. The causality within the explanations originates directly from the cause-effect diagram, which is constructed by hand. [23, p. 612]

While Weld and deKleer admit that Rieger’s vocabulary is richer than those that came before, or within a dozen years after, they take Rieger to task for not building a system that can automatically construct these causal models. Unfortunately, the consequence of this reaction to early work on a rich vocabulary of causal connectives has been the relative abandonment, for over a decade, of work on rich causal vocabularies.

This thesis continues the research on rich causal vocabularies. It addressed Weld and deKleer’s criticism by constructing an inference system that can automatically construct these causal models starting from observations. And rather than rely on common sense examples, where the ubiquity of the knowledge can make intellectually rigorous experiments difficult, this thesis uses a domain where professionals of the highest caliber labor to construct these models, and to justify them to their colleagues.

5.2 Causal Reasoning

Causal reasoning is an old problem in artificial intelligence, and an ancient problem in pre-computational philosophy and psychology. This thesis might be viewed as a computational updating of some of the work by the founders of the scientific method: for example, Francis Bacon and John Stuart Mill.

Doyle’s thesis [5, 6] presents an excellent example of a program that constructs causal models based on observations. Doyle addresses the problem of hypothesizing mechanisms. Given a set of initial events, a set of final events, and a library of mechanisms, his program

hypothesizes a mechanism graph—where nodes are events and arcs are mechanisms—that connects the initial events with the final states. Doyle has abstracted, from the mechanisms in his library, a set of constraints such as type, magnitude, direction and bias of effect. For every gap or inconsistency in an explanation, the signature—in terms of these constraints—of the gap or inconsistency suggests which mechanisms might fill the gap or resolve the inconsistency. Some of these signatures can suggest branched mechanisms such as enablements, cyclic mechanisms, or mechanisms with hidden state. His program includes a simplicity metric on mechanism graphs, so that linear mechanisms are tried before branched ones, acyclic ones before cyclic ones, and ones without hidden state before ones with hidden state.

One of the main differences between the work presented in this thesis and Doyle’s work is that Doyle’s program uses a detailed representation of events, and a library of component mechanisms. This thesis uses a very simple model of events, and it does not rely on a library of component mechanisms. Therefore, the system presented in this thesis can be used in domains where the reasoners have very little prior domain knowledge.

Borchardt’s thesis [3] presents an excellent example of a program that reasons in detail about causal models. Borchardt addresses the problem of comprehending informal causal explanations. He interprets this problem as the task of filling the gaps in an informal causal explanation through the process of retrieving causal associations from a database of precedents, and matching, abstracting or analogizing these precedents to fill the gaps in the current explanations. He represents the steps in a mechanistic explanation as associations between transitions of the values of physical parameters, such as position, speed and direction.

One of the main differences between the work presented in this thesis and Borchardt’s work is that this thesis uses a much simpler model of events than Borchardt’s program, but it uses a much more detailed model of causal connectives. (Borchardt’s program’s only causal connective is an implied “cause”.)

Another difference is that the Borchardt’s program accepts as inputs causal models (to be refined), whereas the program in this thesis accepts as input experimental observations.

The research discussed above all involves the construction of explicit causal models given input data with explicit temporal ordering.

There is another field of research that is concerned with imputing causality to non-causal descriptions of systems. For example, Pearl’s causal graphs [13] provide a formalism for representing conditional probabilities, and techniques for probabilistically inferring causal models from statistical data. The main difference between the research in this thesis and that statistical work is that this work attempts to model the reasoning of biologists who attempt to reduce the uncertainty in their models by conducting carefully controlled experiments. The reasoning formalized in this thesis captures the causal reasoning done by biologists better than does statistical inference.

In the philosophical and AI literatures, there is a lot of discussion of attempts and difficulties of reasoning from counterfactuals to causal statements [18, 19]. The research in this thesis is conducted in the domain of reasoning from controlled, scientific experiments to causal models. None of the philosophical problems dwelled on by the AI research referenced above are relevant to this task.

5.3 Representation and Reasoning in Molecular Biology

Karp’s thesis [8, 9] presents the most detailed example in the literature of a computational analysis of reasoning in experimental biology. Karp describes a rule-based discrete event simulator for the molecular biology domain, and an hypothesis formation algorithm that attempts to reconcile the results of an experiment with the theoretically predicted outcome by suggesting changes to the theory (i.e. the process rules), or to the initial conditions of the experiment.¹ Karp uses these programs to model a fragment of a reconstruction of an important biological discovery. His reconstruction is quite detailed and covers many man-years of biology; his programs were run on only a small part of this reconstruction.

Given a theory and a set of initial conditions for an experiment, his simulator can perform a simulation and generate a final state for the system, and an event trace. If this final state disagrees with the experimental results, his hypothesis formation algorithm can use a set of operators to modify the theory or the initial conditions to resolve the disagreement. The program first regresses undesirable final states back through a process

¹Although resolving contradictions by changing your mind about the facts of the initial conditions of your experiment might seem like cheating, it is an important part of scientific reasoning in this technically complex domain.

trace and then uses design operators to fix the theory, or the initial conditions, in order to avoid the undesirable final state. The program's theory modification operators can add, prevent or remove assertions, or increase or decrease quantities.

For example, if the simulation predicts that a product will be present in the final state, but none is observed, the preconditions for the process that generated the product might be modified so that the process would not be predicted to occur, and therefore the prediction of the product's presence in the final state would not arise. Karp presents a set of design operators that can be used to regress undesired final states back through process traces to generate changes to the process preconditions or to the initial conditions.

The main difference between the work in this thesis and Karp's work is that the work in this thesis uses a much simpler event model, but a more detailed vocabulary of causal connectives.

Chapter 6

Future Directions

This chapter describes several future directions for this research, including designing more sophisticated data interpretation functions, improving the control of reasoning in the inference system, and augmenting the causal reasoning system with background knowledge.

6.1 Qualitative interpretation of experimental data

One area for future research involves the component of the system that converts raw data into qualitative observational statements. The current version of this component of the system performs pairwise comparisons of the values within a single column within a given dataset, and concludes that one value is significantly greater than a second value if they differ by a factor of two. The system also concludes that a small, non-zero value is approximately zero if it is less than or equal to the least value in the column, and if it is less than one thirtysecond of the greatest value in the column.

Although these simple techniques serve adequately for the research presented in this thesis, there are several directions in which they could be improved. First, a more flexible threshold function could be devised. A threshold function might consider several additional factors—such as the maximum and minimum values observed for the given type of measurement in all of the experiments that the system has seen, and the theoretical maximums and minimums for this measurement—in deciding if a given measurement should be judged as indicating the occurrence, non-occurrence or partial occurrence of the relevant event.

Second, the program could reason across experiments in processing raw data. For ex-

ample, the program could fill-in missing controls in a given experiment by using the data in other, similar experiments.

6.2 Control of Reasoning

For the information retrieval task that motivates this thesis, the requirements for the inference system are: that it derives the appropriate causal conclusions from given experimental data, and that it does not derive any incorrect conclusions from the data. It is not required of the system that it only derive the minimum set of conclusions from the experimental data. The program fires all of its rules in a forward chaining fashion, and generates all of the conclusions that can be inferred from the data. This guarantees that the program will not miss a conclusion that could be inferred using the available rules and data, but it also leads to an “overgeneration” of conclusions: i.e., the generation of a large number of true but uninteresting conclusions.

Another direction for future research would be to augment this system with control knowledge so that the system can generate a reduced set of conclusions that correspond to the major conclusions that would be identified by a biologist, as shown, for example, in Figures 4.8 through 4-10. The next sections describe several approaches to this goal.

6.2.1 Inferring Causal Statements with Conjunctions of Events

One of the causes of the overgeneration of causal statements is the generality of the event matcher. The matcher will consider any conjunction of events as a single event for the purpose of firing an inference rule. That is, the matcher is programmed to match an event variable against all possible conjunctions of events. For example, if an inference rule has patterns that match two events, and the matcher is matching the rule against an experimental trial that contains five causative events, the matcher will consider all possible combinations for matching subsets of the five causative events to the two event variables. This can generate a large number of conclusions. The system could be modified to generate a reduced set of conclusions. Some heuristics for achieving this goal are presented in Sections 6.2.3 and 6.2.4.

6.2.2 Inferring Negative Causal Statements

Another cause of overgeneration is the generation of negative causal statements (e.g., the statement that the occurrence of event A does *not* cause the occurrence of event B). As with conjunctions, although there may be important negative conclusions that we would like the program to derive, the uncontrolled generation of all possible negative conclusions leads to a large number of superfluous conclusions.

There are several possible techniques for controlling the inference of negative causal statements. One heuristic involves only inferring a negative conclusion if that conclusion is in some way surprising or informative. A negative statement might be particularly informative in cases where there is a similar positive causal statement. For example, if A causes B, and A and C are similar events, then it might be particularly interesting to infer that C does not cause B (if that is indicated by the experimental evidence). To cite an example from [21] if the addition of calcium ion has a certain effect, then it is interesting to know that the addition of magnesium ion does not have that effect, since both calcium and magnesium ions are similar. (They both have a charge of +2.)

6.2.3 Guiding Inference using knowledge about specific causes

There are several types of domain knowledge that could be used to guide the inference process. One heuristic might involve using knowledge about specific causitive agents to guide which conclusions are inferred. For example, consider the following qualitative observations:

```
(leads-to A B)
(not (leads-to nil B))
(leads-to (conj A C) B)
(not (leads-to C B))
```

From this input, the system can generate the following conclusions regarding C:

```
(not (causes C B))
(not (blocks C A B))
```

The first conclusion is probably more interesting in cases where C is the type of event that is generally stimulatory in the system under study. The addition of Concavilin A

would be such an event for the T-cell activation system discussed in this thesis. The second conclusion is probably more interesting in cases where C is the type of event that is generally inhibitory in the system under study. The addition of Dexamethasone would be such an event for the T-cell activation system.

6.2.4 Guiding Inference using knowledge about general causal models

Background knowledge about causal models in related biological systems can also be used to constrain the generation of causal model statements.

For example, in many cell-signaling systems, signals propagate: (1) from extracellular signal molecules, (2) to cell-surface receptors, (3) to cytoplasmic second messengers, and, finally, (4) to nuclear effectors. A reasoning system augmented with this knowledge can be constrained to generate fewer superfluous causal connections.

With any of these pruning techniques, there is always the possibility that the augmented system will miss a novel causal conclusion. Therefore, although these augmented systems will be useful for generating summaries, it will probably be desirable to use an unfiltered forward reasoning system—of the type presented in this thesis—to test for novel causal conclusions.

6.3 Reasoning with Analogies, Generalizations and Correlations

Another future direction involves using background knowledge to augment pure causal reasoning. For example, causal hypotheses can be generated by examining causal models of analogous biological systems. This section describes several heuristics that can be used to suggest causal models.

Most of these heuristics can be viewed as instances of Occam's razor. They reflect the preference for simpler causal models rather than more complex ones, and for minimizing the number of causal model statements that are needed to account for all cellular processes.

6.3.1 Inferring analogous function from rescue experiments

One common type of experiment in molecular cell biology is the “knockout and rescue” experiment. In this experiment, a biologist deletes a specific cellular component from a cell, and then observes the effects of this deletion. For example, the biologist might test whether any of the normally occurring cellular processes are disrupted by the deletion of this component. The biologist then tries to restore these disrupted processes by adding a new component to the cell. If the presence of this new component can rescue the effects of the initial deletion, then the biologist might hypothesize that these two components (the deleted one and the added one) have analogous functions.

Tanaka et al. [20] present an example of this type of reasoning. In a mammalian cell, GAP (GTPase Activating Protein) is necessary for the conversion of ras-GTP to ras-GDP. In yeast cells, this conversion is observed to occur under normal conditions. If the *ira1* gene is deleted from a yeast cell, preventing the expression of IRA1 protein, then this conversion does not occur. Adding GAP to the yeast cell rescues the block of this conversion that is caused by the deletion of *ira1*. From this experimental result the authors conclude that IRA1 has an analogous function to that of GAP. And, since GAP is necessary for the conversion of ras-GTP to ras-GDP in mammalian cells, they conclude that IRA1 is necessary for this conversion in yeast cells.

6.3.2 Inferring specific pathways from generalized pathways

Another common reasoning technique involves reasoning from general cellular causal models to specific causal models. Many of the components of a cell are members of a few large classes. For example, a cell synthesizes thousands of different species of RNA. And most of the cellular mechanism involved in the synthesis of a given species of RNA are involved in the synthesis of all of the species of RNA.

Depper et al. [4] present an example of this type of reasoning. The drug Actinomycin D prevents all RNA synthesis. Therefore, it prevents the synthesis of the RNA species that corresponds to the protein IL2R. And since Actinomycin D blocks the pathway from PHA to IL2R, therefore the synthesis of IL2R RNA is very likely necessary for the pathway from PHA to IL2R. (In biological terms, the pathway from PHA to IL2R requires *de Novo* IL2R RNA synthesis.) Note that although it is logically possible that Actinomycin D blocks the

pathway from PHA to IL2R by preventing the synthesis of some RNA species other than IL2R, a biologist would consider this causal model less likely than the causal model that states that Actinomycin D blocks IL2R by preventing IL2R RNA synthesis.

6.3.3 Inferring common causes from correlations

Biologists frequently use circumstantial evidence, such as observed correlations, to hypothesize causal models. For example, if (i) two events involve cellular components of the same class, and (ii) the events are caused by the same external event, and (iii) they are blocked by the same external event, then a biologist might hypothesize that: (a) these two events have a common upstream event; (b) that this upstream event causes both of these two events; (c) that this upstream event is itself caused by the external event that causes these two events; and (d) that this upstream event is blocked by the external event that blocks these two events.

Wiskocil et al. [25] present an example of this type of reasoning. Since PHA causes both IL2 RNA synthesis and IFN γ RNA synthesis, and since Cyclosporin A blocks both of these pathways, perhaps there is a common regulatory event that controls the synthesis of both IL2 RNA and IFN γ RNA.

6.3.4 Restricting generalizations with negative data

The general predilection for simplifying causal models must be controlled by experimental tests. This example is taken from [25], and continues the example presented in the previous section. Since Cyclosporin A blocks the synthesis of IL2 RNA and IFN γ RNA, perhaps it blocks all RNA synthesis? This is an appealing hypothesis because it would simplify the causal model, by explaining two phenomena—IL2 RNA block and IFN γ RNA block—with one mechanism—RNA synthesis block.

However, the observation that Cyclosporin A does not block the synthesis of Actin RNA disproves this hypothesis. The conclusion from this data is that IL2 RNA synthesis and IFN γ RNA synthesis must involve events that are not involved in Actin RNA synthesis. This conclusion enhances the specificity of Cyclosporin A's effect, by showing that Cyclosporin A only effects IL2 RNA and IFN γ RNA, and not all RNA. This in turn enhances the hypothesis that a common event controls both IL2 RNA and IFN γ RNA.

6.4 Building intelligent research assistant programs

This thesis has presented a foundation for building programs that can reason from the experimental data published in biology journals to causal models of biological systems. These programs could provide literature retrieval based on understanding the published experiments and their ramifications. Early versions of these programs could enhance the capabilities of current retrieval systems. More advanced versions of these programs could develop into full-fledged intelligent research assistants. Building these systems will advance the progress of biology, and will advance our understanding of how we reason about the world.

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Appendix A

Experimental Observations

This appendix contains the input data for the experiments described in Chapter 4. This data is excerpted from [15, 21, 11].

additions	TAC	3H-TDR	IFN
—	1	1.0	0.0
CON-A	5	11.0	0.8
EXOG-IL2	1	—	0.0
CON-A + EXOG-IL2	56	300.7	6.0
CON-A + DEX	1	0.2	0.2
CON-A + EXOG-IL2 + DEX	26	88.8	6.3
CON-A + EXOG-IL2 + ANTI-TAC	20	73.9	2.9
CON-A + ANTI-TAC	6	0.6	0.9

Table A.1: Reen&Yeh Table 1

additions	TAC	IFN
—	1	4
CON-A	18	24
TPA	3	4
CON-A + TPA	42	256
EXOG-IL2	1	4
CON-A + EXOG-IL2	48	192
TPA + EXOG-IL2	29	4
CON-A + TPA + EXOG-IL2	54	512

Table A.2: Reem&Yeh Table 2

additions	3H-TDR	IFN
CON-A + TPA	63.8	2048
CON-A + TPA + ANTI-TAC	7.9	512
CON-A + TPA + EXOG-IL2 + ANTI-TAC	4.5	512

Table A.3: Reem&Yeh Table 3

additions	TAC	3H-TDR	IFN
CON-A + TPA	62.3	55.0	7.0
CON-A + TPA + EXOG-IL2	80.1	79.9	13.7
CON-A + TPA + ANTI-TAC	40.9	16.4	3.2
CON-A + TPA + EXOG-IL2 + ANTI-TAC	47.6	28.0	6.1

Table A.4: Reem&Yeh Table 4

additions	TAC	3H-TDR	IFN
CON-A + TPA	42	113.1	11.4
CON-A + TPA + DEX	46	79.8	1.5
CON-A + TPA + ANTI-TAC	39	42.0	6.9
CON-A + TPA + DEX + ANTI-TAC	7	19.8	0.7

Table A.5: Reem&Yeh Table 5 Experiment 1

additions	TAC	3H-TDR	IFN
CON-A + TPA	54	134.0	108.4
CON-A + TPA + EXOG-IL2	90	224.1	115.5
CON-A + TPA + DEX	48	24.5	16.3
CON-A + TPA + ANTI-TAC	32	78.1	44.8
CON-A + TPA + ANTI-TAC + DEX	10	4.6	5.7
CON-A + TPA + ANTI-TAC + DEX + EXOG-IL2	27	37.1	37.5

Table A.6: Reem&Yeh Table 5 Experiment 2

additions	TAC	IFN
CON-A + TPA	41	256
CON-A + TPA + EXOG-IL2	82	256
CON-A + TPA + CSA	1	4
CON-A + TPA + EXOG-IL2 + CSA	22	4

Table A.7: Reem&Yeh Table 6

additions	3H-TDR
—	0
IONO	0
TPA	0
IONO + TPA	8
A23187	0
A23187 + TPA	9
CON-A	5
CON-A + TPA	9

Table A.8: Truneh Figure 1

additions	3H-TDR
TPA	0
IONO + TPA	10
TPA + EGTA	0
IONO + TPA + EGTA	0
TPA + EGTA + CACL2	0
IONO + TPA + EGTA + CACL2	12

Table A.9: Truneh Figure 2

additions	IL2
IONO	1
CON-A	21
IONO + TPA	22
CON-A + TPA	27

Table A.10: Truneh Figure 3

additions	IL2R
—	4
IONO	4
CON-A	8
TPA	4
IONO + TPA	11
CON-A + TPA	2

Table A.11: Truneh Figure 4

additions	3H-TDR
—	316
IONO	620
CON-A	901
EXOG-IL2	26365
TPA	2638
IONO + TPA	5940
CON-A + TPA	2638

Table A.12: Truneh Table 1

additions	INCR-CA
—	0
EXOG-IL2	0
PHA	140

Table A.13: Mills Table 1

additions	INCR-CA
—	0
EXOG-IL2	0
CON-A	106

Table A.14: Mills Table 2

additions	3H-TDR
—	4690
EXOG-IL2	100065
EGTA	1500
EXOG-IL2 + EGTA	46540

Table A.15: Mills Table 3