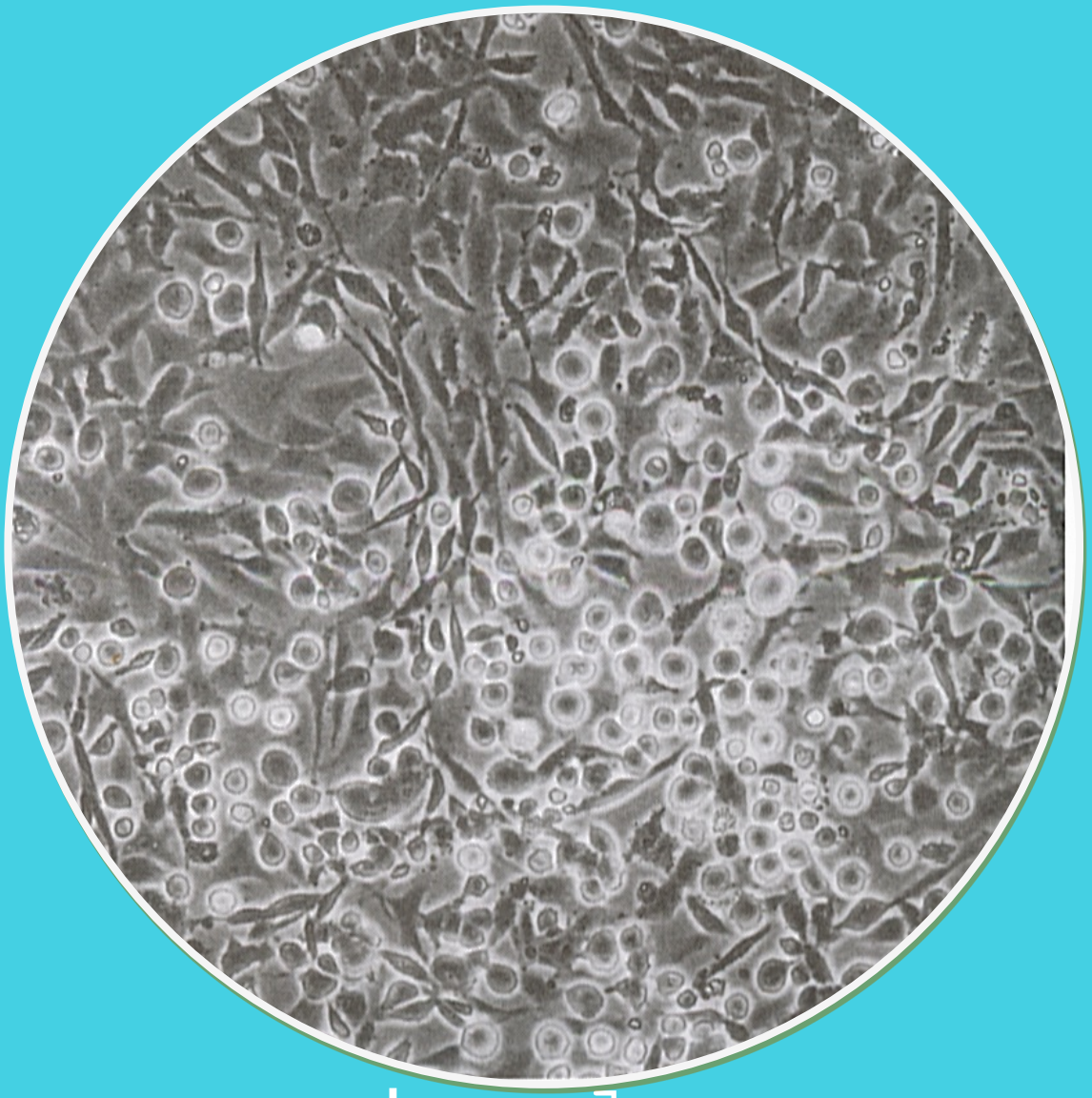

# Can a single gene cause cancer?

## Key Stage 3 Programme

| | |
|---|---|
| Pupil Name | |
| Handbook Designed by | Dr. J. Bell |

# Timetable and Assignment Submission

## Timetable – Tutorials

| Tutorial | Date | Time | Location |
|---|---|---|---|
| 1 | December 12, 2018 | 2:00pm | King's College, London |
| 2 | January 11, 2018 | 3:10pm | Ark William Parker |
| 3 | January 18, 2019 | 3:10pm | Ark William Parker |
| 4 | January 25, 2019 | 3:10pm | Ark William Parker |
| 5 | February 1, 2019 | 3:10pm | Ark William Parker |
| 6 (Feedback) | February 22, 2019 | 3:10pm | Ark William Parker |
| 7 (Feedback) | March 22, 2019 | 3:10pm | Ark William Parker |

## Timetable – Homework Assignments

| Homework Assignment | Description | Due Date |
|---|---|---|
| Tutorial 1 | Write 500 words on "How DNA works" | January 8, 2019 |
| Tutorial 2 | Write notes on animal cells and how they divide | January 18, 2019 |
| Tutorial 3 | Write notes on how cell cultures and viruses can be used in research | January 25, 2019 |
| Tutorial 4 | Research and make notes on causes of cancer | February 1, 2019 |
| Tutorial 5 | Describe an experimental model that could test whether a single gene can cause cancer and discuss the implications for the prevention of cancer (2,000 word dissertation) | February 15, 2019 (draft)<br>March 8, 2019 (final) |

## Assignment Submission – Lateness and Plagiarism

| **Lateness** | |
|---|---|
| Submission after midnight on March 8th | 10 marks deducted |
| **Plagiarism** | |
| Some plagiarism | 10 marks deducted |
| Moderate plagiarism | 20 marks deducted |
| Extreme plagiarism | Automatic fail |

# Uni Pathways – Pupil Feedback Report

| Grade | Marks | Guidance |
|---|---|---|
| 1st | 70+ | |
| 2:1 | 60-69 | |
| 2:2 | 50-59 | |
| 3rd | 40-49 | |
| Working towards a pass | 0-39 | |
| Did not submit | DNS | No assignment received by The Brilliant Club |

| Lateness | |
|---|---|
| Any lateness | 10 marks deducted |
| **Plagiarism** | |
| Some plagiarism | 10 marks deducted |
| Moderate plagiarism | 20 marks deducted |
| Extreme plagiarism | Automatic fail |

| | | | |
|---|---|---|---|
| **Name of PhD Tutor** | Dr. J. Bell | | |
| **Title of Assignment** | Describe an experimental model that could test whether a single gene can cause cancer, how such a gene could work, and discuss the implications for the prevention of cancer | | |
| **Name of Pupil** | | | |
| **Name of School** | Ark William Parker Academy | | |
| **ORIGINAL MARK / 100** | | **FINAL MARK / 100** | |
| **DEDUCTED MARKS** | | **FINAL GRADE** | |

If marks have been deducted (e.g. late submission, plagiarism) the PhD tutor should give an explanation in this section:

**Learning Feedback Comment 1 -** *How DNA works*

| What you did in relation to this Key Learning Priority | How you could improve in the future |
|---|---|
| *Enter feedback here* | *Enter feedback here* |

| Learning Feedback Comment 2 – *Cells and cancer* | |
|---|---|
| **What you did in relation to this Key Learning Priority**<br><br>*Enter feedback here* | **How you could improve in the future**<br><br>*Enter feedback here* |

| Learning Feedback Comment 3 – *Scientific experiments* | |
|---|---|
| **What you did in relation to this Key Learning Priority**<br><br>*Enter feedback here* | **How you could improve in the future**<br><br>*Enter feedback here* |

| Resilience Comment | |
|---|---|
| **How you showed learning resilience during the course**<br><br>*Enter feedback here* | **How you could build learning resilience in the future**<br><br>*Enter feedback here* |

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# Course Rationale

Around one in two people are likely to be diagnosed with cancer over the course of their lifetime. Although many of these cases are treatable, it is highly likely that everyone will be affected by cancer either directly or indirectly through another family member at some point in their lifetime.

What does this disease involve?

How does it arise?

What can be done to prevent it?

How can science contribute to understanding it?

We will be looking at the answers to these questions and others, and along the way we will be looking at many topics that are covered in the GCSE and A level Biology courses, such as:

DNA

Genes

Cells

Mutations

By the end of this course you should have an understanding of the biology of cancer, and the rationale of cancer research, and you should be in a position to evaluate research findings in the field.

# Mark Scheme

STEM Mark Scheme

| Skills | 1st (70-100) | 2:1 (60-69) | 2:2 (50-59) |
|---|---|---|---|
| **Knowledge and Understanding** | • All content included is relevant to the general topic and to the specific question/title<br>• Good understanding of all the relevant topics.<br>• Scientific terms are defined and used accurately throughout<br>• Clear justification on how the content included is related to the specific issues that are the focus of the assignment | • Most of the content included is relevant to the general topic and to the specific question/title<br>• Good understanding of most the relevant topics<br>• Scientific terms are used accurately but not always clearly defined.<br>• Adequate justification on how the content included is related to the specific issues that are the focus of the assignment | • Some of the content included is relevant to the general topic and to the specific question/title<br>• Good understanding on some of the relevant topics but occasional confusion on others.<br>• Scientific terms are used mostly accurately with occasional confusion and often not defined.<br>• Some justification on how the content included is related to the specific issues that are the focus of the assignment |
| **Critical Evaluation / Problem solving** | • Moved beyond description to an assessment of the value or significance of what is described<br>• Evaluative points are consistently explicit/systematic/reasoned/justified<br>• Effective critiques on the reliability of sources provided<br>• Consistently demonstrate clear, analytical and logical steps to solving problems | • Mostly description but some assessment of the value or significance of what is described<br>• Evaluative points are mostly explicit/systematic/reasoned/justified<br>• Some evidence of critiques on the reliability of sources provided<br>• Some examples of solving problems but not consistently clear, analytical and logical | • Only description with minimal assessment of the value or significance of what is described<br>• Evaluative points are at times explicit/systematic/reasoned/justified<br>• Limited evidence of critiques on the reliability of sources provided<br>• Demonstrate clear steps to solving problems but not consistently analytical or logical |

| | | | |
|---|---|---|---|
| **Structure and Presentation** | • Ideas are presented in paragraphs and arranged in a logical structure that is appropriate for the assignment<br><br>• The introduction clearly outlines how the essay/report will deal with the issues<br><br>• The conclusion summarises all the main points clearly and concisely<br><br>• All calculations, formulas and methods are clearly structured, clear to follow and correct<br><br>• Tables and graphs are effectively constructed including appropriate headings, units and scales.<br><br>• All sources are referenced correctly in an agreed format | • Ideas are presented in paragraphs and arranged in a structure that is mostly appropriate for the assignment<br><br>• The introduction adequately describes how the essay/report will deal with the issues<br><br>• The conclusion summarises most of the main points clearly<br><br>• Calculations, formulas and methods are mostly structured, clear to follow and correct<br><br>• Most tables and graphs are well constructed<br><br>• Most sources are referenced correctly in an agreed format | • Ideas are presented in paragraphs and arranged in a structure<br><br>• The introduction mentions how the essay/report will deal with the issues<br><br>• The conclusion summarises some of the main points clearly<br><br>• Calculations, formulas and methods are not always structured, clear to follow and correct.<br><br>• Some tables and graphs are well constructed but contains some errors<br><br>• Some sources are referenced correctly in the agreed format with occasional errors |

# Glossary

This is so that you can look up the meaning of words as you come across them. You do not have to memorise all the definitions!

| Word | Definition |
|---|---|
| Amino acid | The parts that, joined together, make a protein. There are twenty different amino acids. |
| Base | Part of a DNA molecule. There are 4 different ones, A, T, C and G, and the order of them in the DNA molecules can determine which proteins are made. |
| Cancer | A disease characterised by uncontrolled multiplication of a single cell causing tumours. |
| Carcinogen | A substance that causes cancer. |
| Cell | The basic unit of living organisms. Some organisms are made out of only one cell, but the human body is made out of many billions of cells. |
| Cell culture | A way of growing cells in plastic dishes in a liquid medium containing all the food they need. |
| Cell cycle | A series of stages that cells pass through allowing them to divide and produce new cells. |
| Cell division | The process by which cells make a copy of themselves in order to become more numerous. |
| Chromosome | A long piece of DNA, containing many genes. In human cells there are 23 pairs of chromosomes, 46 in all, and proteins wrap around the DNA. |
| DNA | Deoxyribonucleic acid. A long and complicated molecule that contains the information required to make proteins. It contains 4 'bases', A,T,C and G. The order of these determines which proteins are made. |
| DNA synthesis | The process by which DNA is copied before new cells are made. |

| | |
|---|---|
| Gene | A section of DNA that contains the information to make one protein. |
| Molecule | A combination of multiple atoms joined together. Biological molecules are very complicated. |
| Mutagen | A substance that can change DNA, and so change a gene so that it gives instructions for a protein that is different from the one that it usually codes for. |
| Mutation | A change in DNA that can make a gene code for a protein other than the one it usually does. |
| Nucleus | The part of a cell that contains the DNA. |
| Oncogene | A gene that causes cancer. |
| Protein | A complex molecule made from any combination of 20 different amino acids. There are practically an indefinite number of different possible protein molecules. |
| Regulatory gene | A gene that gives instructions to make a protein that can switch other genes on or off. |
| RNA | Ribonucleic acid. A molecule similar to DNA that is used to deliver information from a gene to the place in cells where proteins are made. |
| Src (pronounced "sarc") | An example of an oncogene; the first oncogene to be discovered. It causes tumours in birds. |
| Temperature sensitive mutant | A mutant gene that makes a correct protein at one temperature and one that doesn't work at another temperature. |
| Transformation | Changing normal cells into cancer cells. |
| Tumour | A mass of cells that do not form a normal part of the body. |
| Virus | A small piece of DNA or RNA surrounded by protein which can get inside cells and sometimes add genes to them. Viruses can not live on their own. |

# Knowledge Organiser

This summarises the key knowledge needed in the course. Don't worry if it doesn't make sense on its own – it needs explaining! Key words given in the glossary above are written in **bold** the first time they occur.

The human body is made of **cells**, the basic units of all living things (Fig. 1). It starts with one cell which continues to divide, producing more and more cells until the body is fully grown. Then the cells are supposed to stop dividing. If they start dividing again by mistake then a **tumour** is formed which can lead to **cancer**.

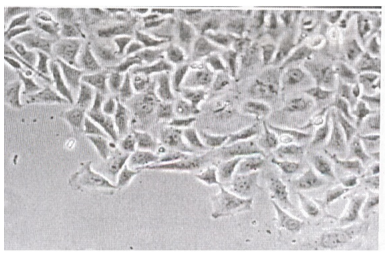
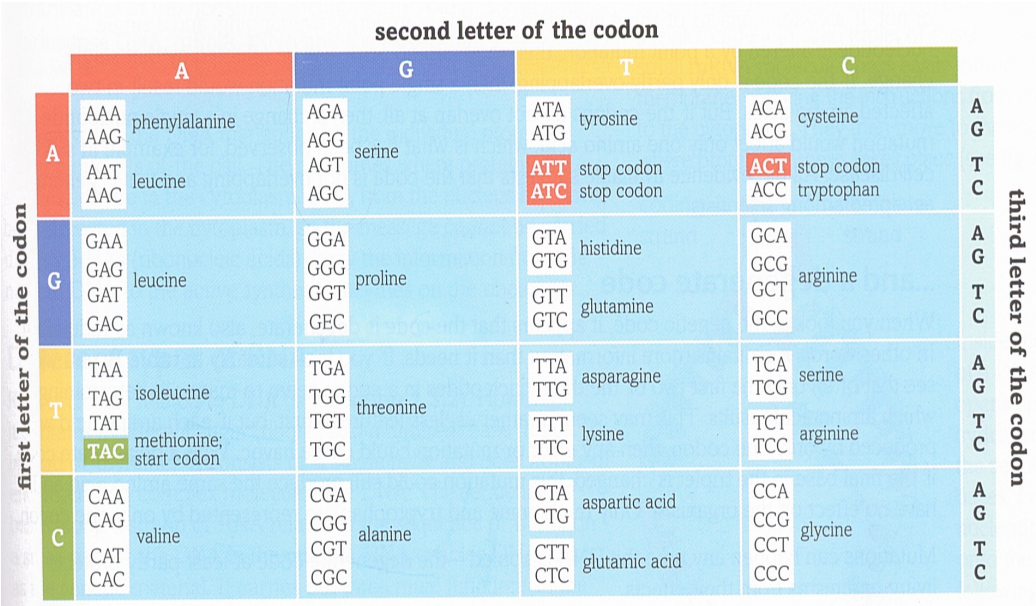


Fig. 1 Animal cells in culture seen under a microscope

The information that determines what the cells and the body are like is contained in **DNA**. The DNA is kept in the **nucleus** of the cell DNA is a very large **molecule** made up of many atoms in a specialised arrangement. Nearly every cell contains DNA and the DNA is identical in nearly every cell.

Before they divide, cells need to double up the quantity of DNA in them so that there is enough for each new cell. The information in the DNA is written with four letters A, T, C and G that stand for the names of four '**bases**' which are parts of the DNA (Fig. 2). The language of DNA is written in words of three letters. Each word gives the code for an **amino acid**. There are twenty different amino acids, and a long chain of amino acids joined together makes a **protein**. The genetic code is the dictionary giving the correspondence between the base sequence in the DNA and the amino acid sequences in proteins.



| first letter of the codon | second letter of the codon: A | G | T | C | third letter of the codon |
|---|---|---|---|---|---|
| A | AAA, AAG phenylalanine; AAT, AAC leucine | AGA, AGG, AGT, AGC serine | ATA, ATG tyrosine; ATT stop codon; ATC stop codon | ACA, ACG cysteine; ACT stop codon; ACC tryptophan | A, G, T, C |
| G | GAA, GAG, GAT, GAC leucine | GGA, GGG, GGT, GEC proline | GTA, GTG histidine; GTT, GTC glutamine | GCA, GCG, GCT, GCC arginine | A, G, T, C |
| T | TAA, TAG, TAT isoleucine; TAC methionine; start codon | TGA, TGG, TGT, TGC threonine | TTA, TTG asparagine; TTT, TTC lysine | TCA, TCG serine; TCT, TCC arginine | A, G, T, C |
| C | CAA, CAG, CAT, CAC valine | CGA, CGG, CGT, CGC alanine | CTA, CTG aspartic acid; CTT, CTC glutamic acid | CCA, CCG, CCT, CCC glycine | A, G, T, C |

Fig. 2 The genetic code

The DNA stays in the **nucleus** of the cell. DNA is organised into chromosomes. Humans have 23 pairs of chromosomes, 46 in all. Half of them come from the mother, and half come from the father. A **gene** is a length of DNA that contains the information to make one protein. When the gene is 'switched on' that DNA is copied into a **molecule** called RNA, which takes the information to the part of the cells where proteins are made, much as a page in a book in a library could be copied and taken out of the library.

Some genes, called **regulatory genes** make proteins that 'switch on' other genes, causing these genes to send out the information to make proteins. One such gene switches on all the genes that cause cells to divide, when they need to, for example when there is an injury. This type of gene is called a **protooncogene**.

A **mutation** occurs when the order of the letters, A, T, C and G in DNA are changed by accident. When this happens, a gene can give instructions for a different protein. The gene can turn into an **oncogene** which codes for a protein that gives instructions for cell to divide whether that is needed or not, and is called an **oncogene.** This can lead to cancer.

# Tutorial 1 – DNA

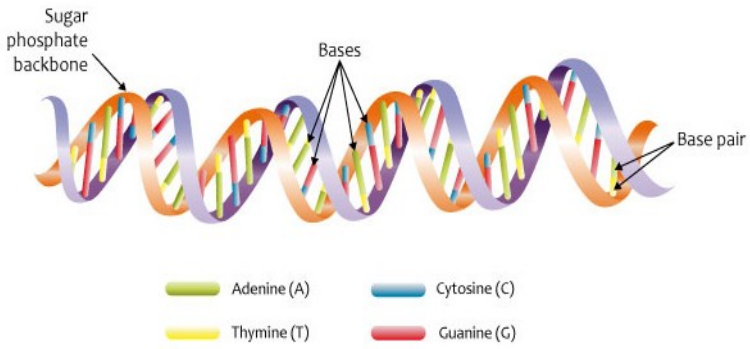



Fig. 1.1 The structure of DNA

## What is the Purpose of Tutorial 1?

- To understand what DNA is
- To explain how DNA provides instructions

DNA works by giving instructions for the assembly of different proteins that are made in a cell from amino acids. The instructions are given a language that has four letters, A, T, C and G, and in words of three letters.

1. How many 3 letter words can you make with these 4 letters? You can use each letter once, twice or three times in a word, or not at all. Note that this includes not only words that exist in English, such as CAT and TAG, but also ones that don't, like ATC and GGG. Use the cards provided to help you with this.

Your answer:

.....

Proteins are made from 20 different amino acids joined together in a chain.

2. Calculate how many different proteins 10 amino acids long you could make from the 20 different amino acids (difficult question)?

.....

3. What are proteins for?

.....

4. What could happen to a protein if the order of the letters A, T, C, and G in DNA was changed?

.....

**Baseline assignment (homework):** Write 500 words on "How DNA works." **Due:** January 8. 2019

# Notes

# Notes

# Tutorial 2 – Cells and cancer

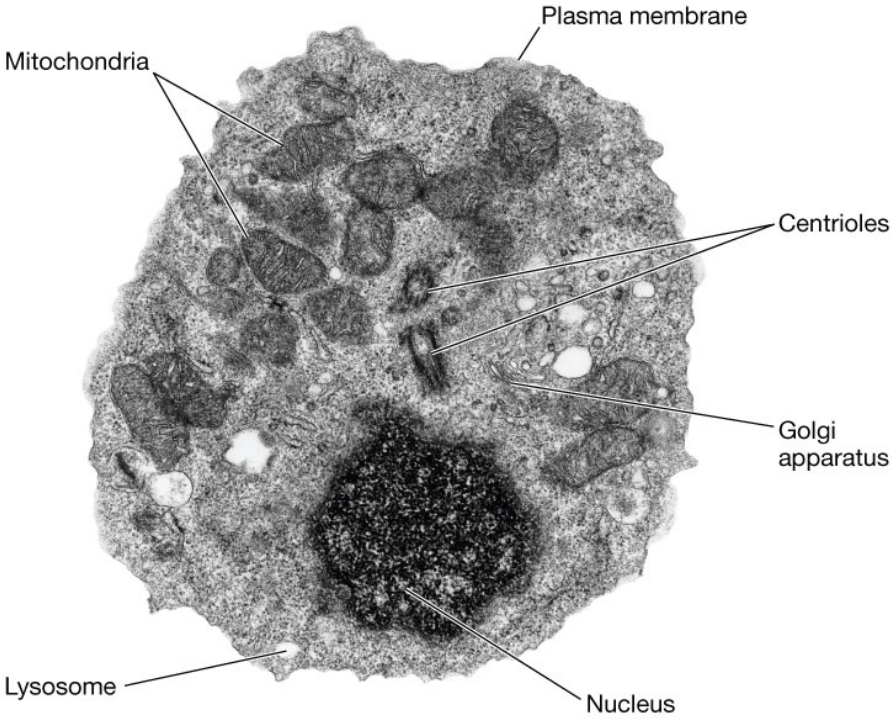



**Fig 2.1 An animal cell**

## What is the Purpose of Tutorial 2?

- To understand what a cell is
- To learn how cells divide
- To consider what happens when cell division goes wrong

1. How many cells are there in the human body?

Write your estimate here.....

2. If cells divide every 12 hours, how many cells would be produced from one cell in two weeks?

.....

3. What do cells need to do in order to divide?

.....

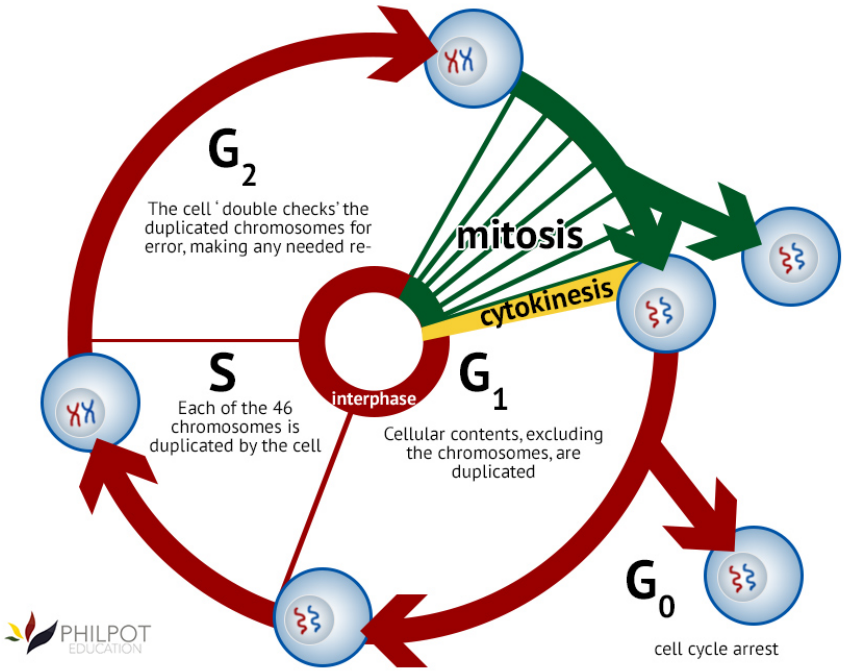
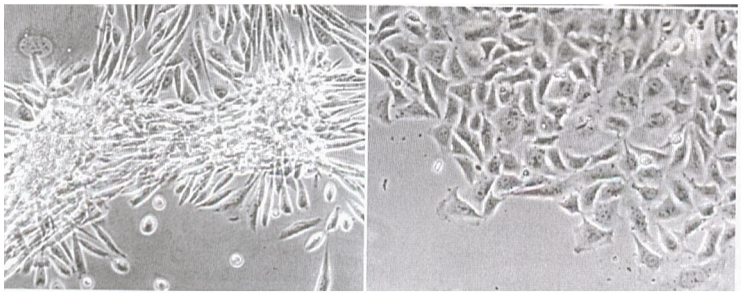



Fig. 2.2 The cell cycle

4. Look at these two pictures of cells. What differences can you see in them?



a b

Fig. 2.3 Transformed animal cells (a) and normal animal cells (b)

Differences:

.....

Challenge question: why do you think the two images look different?

.....

**Homework:** Write notes on animal cells and how they divide. **Due:** January 18, 2019

# Tutorial 3 – Cell culture, viruses, models for research

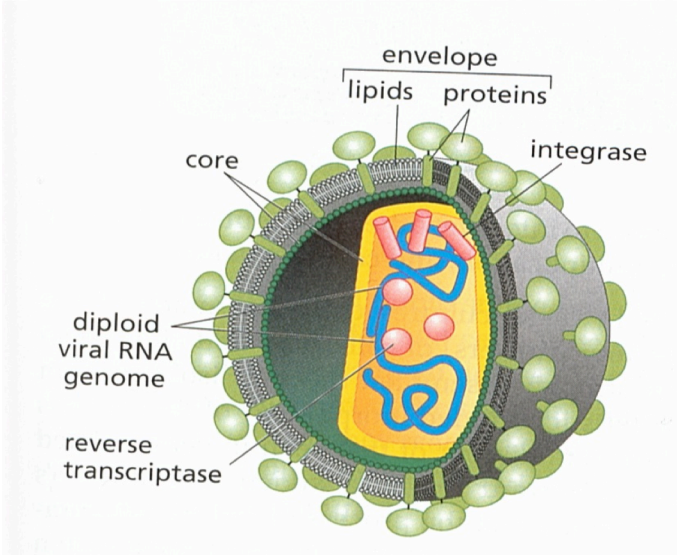



Fig. 3.1 Diagram of the structure of a retrovirus

## What is the Purpose of Tutorial 3?

- To discuss the idea of experimental models
- To give an introduction to how cells can be grown in the lab
- To explain what a virus is and how viruses can be used in research

Scientists can use a 'model' system to carry out an experiment to get the answer to a question.

## Discussion:

1. What do you understand by the word 'model'?

.....

2. Why would you use a model in cancer research?

.....

In this case the model used is chicken cells grown in culture, which are infected with a virus that can put a cancer gene into them.

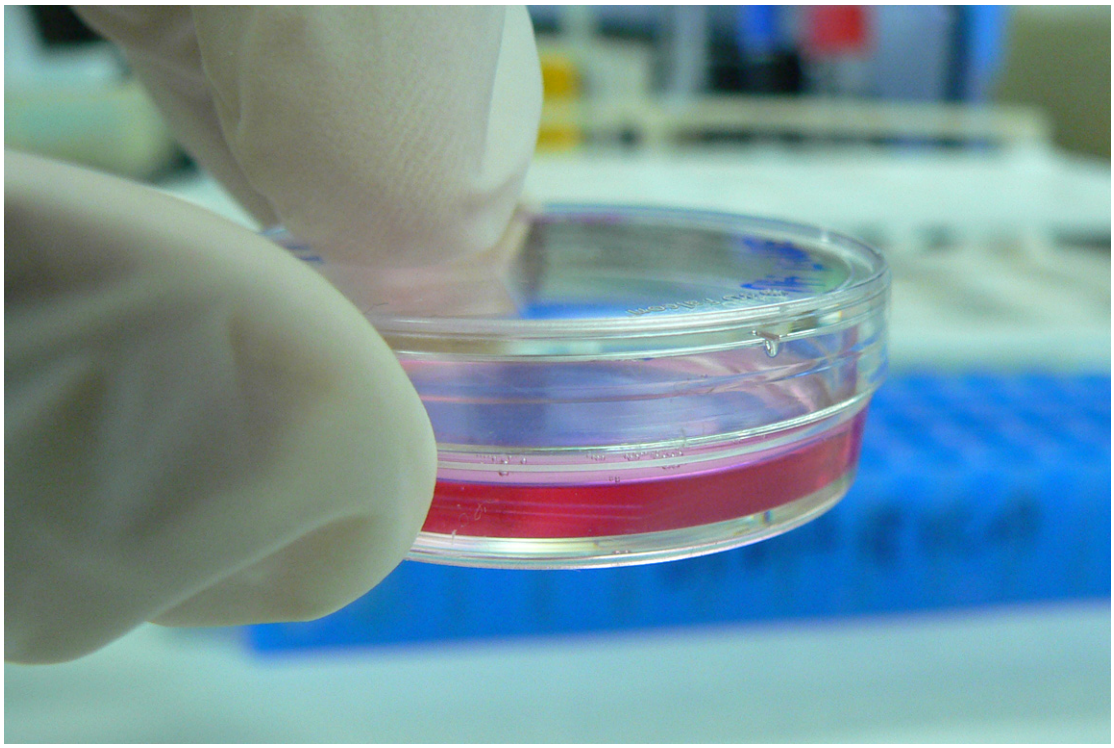


Fig. 3.2 A Petri dish containing animal cells in culture

Cells need food in order to grow. Normally it is delivered by blood vessels. When cells are grown in plastic dishes, instead of in their natural environment, in a body, they can be fed by a liquid medium containing all the nutrients they need.

3. What do you understand by the word virus?

.....

4. How does a virus differ from a cell?

.....

**Homework:** Write notes on how cell cultures and viruses can be used in research. **Due:** January 25, 2019

# Notes

# Notes

# Tutorial 4 – A scientific experiment

## What is the Purpose of Tutorial 4?

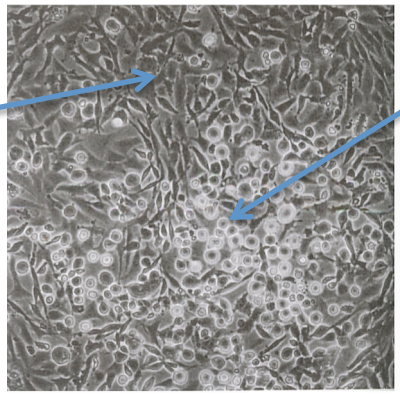
- To give an introduction to the principles of scientific research
- To present an experiment to answer the question "can a single gene cause cancer"

When scientists do an experiment, they change one thing, and keep everything else the same, so they can determine the effect of that one change. For example, if you are determining how fast cells grow, you grow cells in a number of different dishes in identical conditions, but count them after different times.

The body is made of cells that continue to divide until it is fully formed. Then cell division stops.

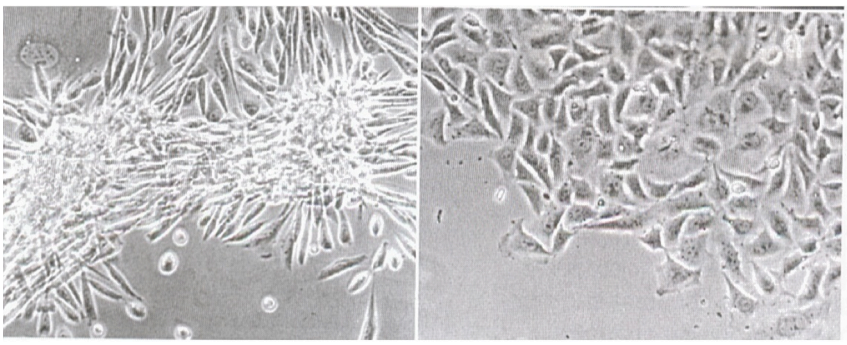
Cancer occurs when a cell continues to divide when it should have stopped.

Rous sarcoma virus makes normal chicken cells turn into cancer cells.




Cancer cells divide and take up sugar more quickly than normal cells. Before each time they divide they make new DNA.

A *temperature sensitive* mutant of the virus 'transforms' cells into cancer cells at 35$^{\circ}$ but not at 41$^{\circ}$. (41$^{\circ}$ is the normal body temperature for birds.)



At 35$^{\circ}$ At 41$^{\circ}$

Cells were cultured at 41$^{\circ}$ in Petri dishes with nutrients, but without any of the 'growth factors' that normally make them divide. They were infected with the mutant virus, then shifted to 35$^{\circ}$. The number of cells was counted at the start and after 29 hours. Sugar uptake and DNA being made was monitored for 10 hours after the shift in temperature.

# Results

1. Discuss one of the three figures showing the results in pairs, and try and work out what conclusions you can draw from it.

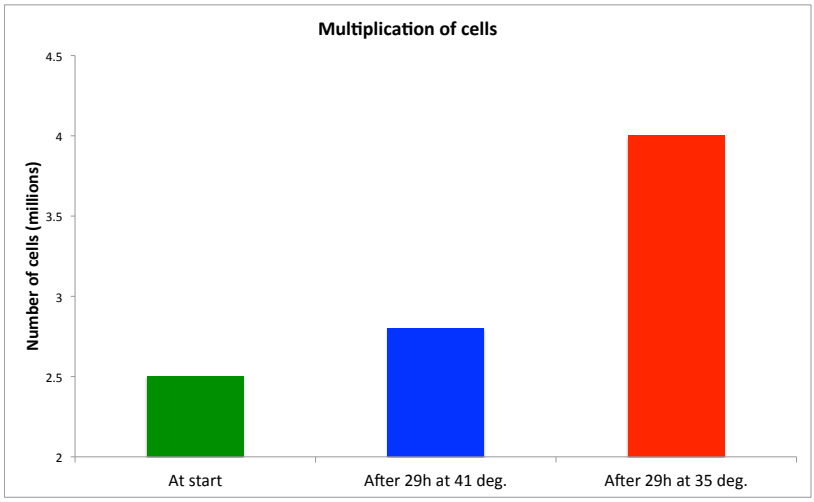



Fig. 4.1 Multiplication of cells infected with a temperature sensitive mutant of Rous sarcoma virus

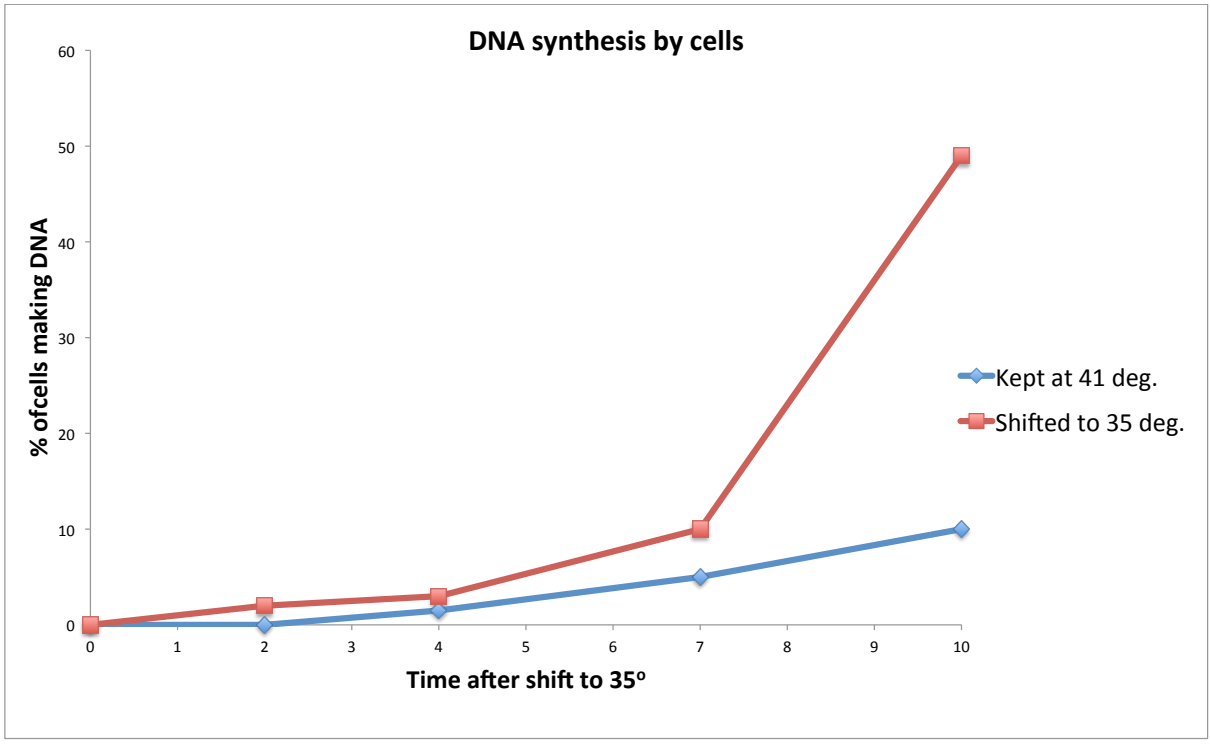



Fig. 4.2 DNA synthesis by cells infected with a temperature sensitive mutant of Rous sarcoma virus

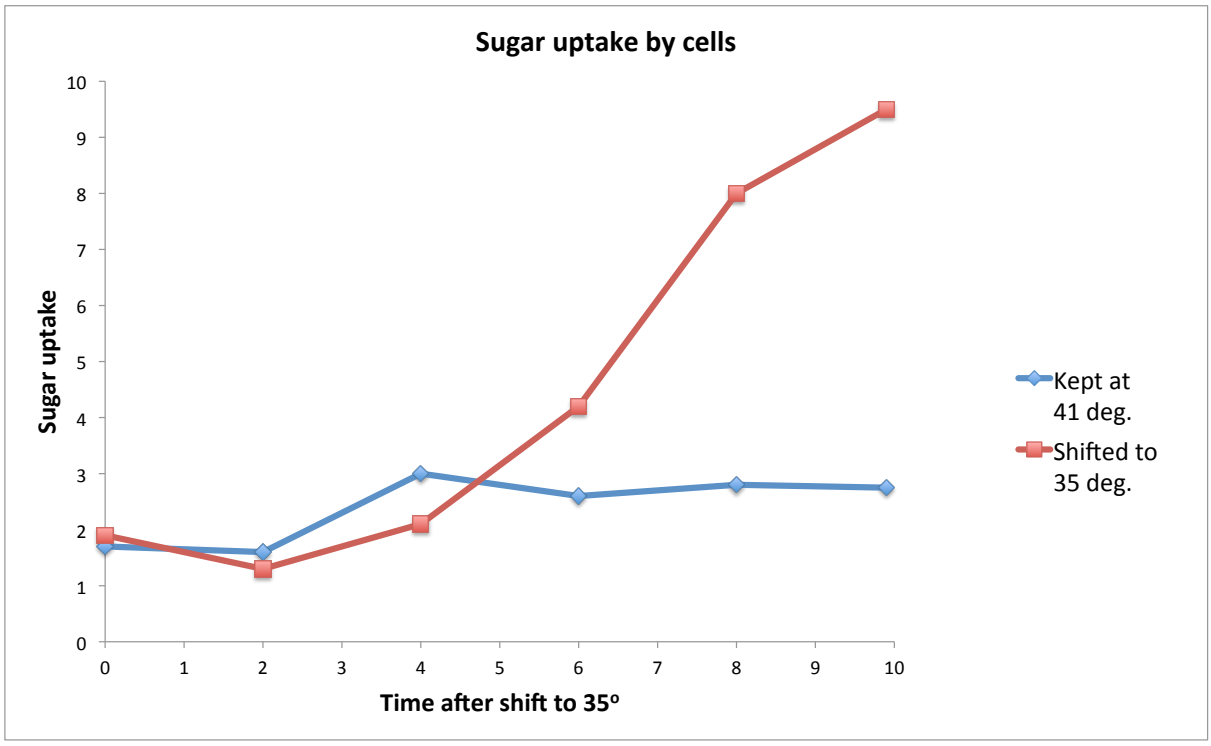



Fig 4.3 Sugar uptake by cells infected with a temperature sensitive mutant of Rous sarcoma virus

**Homework:** Research and make notes on causes of cancer **Due:** February 1, 2019

# Notes

# Notes

# Tutorial 5 – How is cancer caused and how can it be prevented?

## What is the Purpose of Tutorial 5?

- In this tutorial we will be looking at how genes like src could have come about, how they could cause cancer, and what the implications are for the prevention of cancer.

1. Share the results of your research on the causes of cancer

.....

The experiment has shown that one gene is sufficient to cause cancer, but in order for cells to divide many functions have to be carried out, requiring proteins coded for by many genes. So how can one gene cause cell division? The answer is that it must be a **regulatory gene**, one that 'switches on' other genes. A normal gene whose job it is to switch on the genes responsible for cell division *when it is required* has been changed into a gene which switches on the genes for cell division *whether it is required or not.*

## Discussion

2. How could the substances in tobacco smoke, for example, cause cancer?

.....

3. How can cancer be prevented?

.....

**Final assignment:** Describe an experimental model that could test whether a single gene can cause cancer, how such a gene could work, and discuss the implications for the prevention of cancer (2,000 word dissertation).
**Due:** February 15, 2019

Success criteria

A successful dissertation will be written in scientific language (see appendices). It will include the basic biological principles of how DNA determines the structure of proteins, the use of models in scientific research, the principle of how regulatory genes can control a large number of cell functions such as those required for cell division, how mutations can upset the function of a regulatory gene, and thus how mutagens can be carcinogens and cancer can be prevented by avoiding mutagens.

# Notes

# Notes

# Tutorial 6 – Feedback tutorial

## What is the Purpose of Tutorial 6?

- To receive feedback on draft final assignments.
- To improve scientific writing skills
- To discuss technical questions arising from assignments

What did you do well in your draft assignment?

How can you improve your assignment?

**Homework:** Finalise assignment on the topic below. **Due**: March 8, 2019

Describe an experimental model that could test whether a single gene can cause cancer and discuss the implications for the prevention of cancer (2,000 word dissertation).

# Notes

# Notes

# Tutorial 7 – Feedback tutorial

## What is the Purpose of Tutorial 7?

- To receive feedback on final assignments.
- To share examples of best practice with the other pupils in your group.
- To write targets for improvement in school lessons.
- To reflect on the programme including what was enjoyed and what was challenging.

## Final assignment feedback

| What I did well... | What I could have improved on... |
|---|---|
| | |

| My target for future work is... |
|---|
| |

## Reflecting on Uni Pathways

| What did you most enjoy about Uni Pathways? |
| --- |
| |

| What did you find challenging about the programme? | How did you overcome these challenges? |
| --- | --- |
| | |

# Appendix 1 – Principles of scientific writing

There are two main types of scientific writing: reviews, which summarise the different research on a given topic, and research articles, which report research that has been carried out by the authors.

In both cases, the language has to be very precise. For example, you could not write "In China, they eat a bigger amount of meat than in Luxembourg." What would that mean? In China, the population is 1.38 billion, whereas in Luxembourg, it is only 600,000, so of course they eat more meat in China. What is of scientific interest, for example when trying to see if meat eating has an effect on the incidence of cancer, is the quantity eaten on average per person. And you can't say a bigger 'amount' – that is too vague. You would use mass, say in kilogrammes, and you would have to also specify the time, for example, per year. Also you wouldn't use 'average' as technically, that could mean several things. You would express the figures in mean mass of meat eaten, per person per year.

Another thing is that scientific reports are generally expressed in the passive voice. This means that instead of writing "Me and my mate Harry heated up some water," you would write (again being precise) "250ml of water was heated to 45 degrees C." When writing the results of an experiment, it is important to describe it in sufficient detail so that other scientists can do it again to see if they get the same results. Research reports generally start with an introduction, which situates the problem, then a detailed account of what was done, followed by the results, and then finally a discussion of the significance of the results. References (see Appendix 2) are put at the end. See Appendix 3 for an example of a scientific research report.

A review article starts with explaining the overall significance of the topic, for example cancer. Then you start to review the research on the topic, always citing references (see Appendix 2). When you talk about work done by others you always use the last names, for example "Watson and Crick proposed a structure for DNA based on the experimental results of Franklin and Wilkins."

# Appendix 2 – Referencing correctly

When you get to university, you will need to include references in the assignments that you write, so we would like you to start getting into the habit of referencing in your Brilliant Club assignment. This is really important, because it will help you to avoid plagiarism. Plagiarism is when you take someone else's work or ideas and pass them off as your own. Whether plagiarism is deliberate or accidental, the consequences can be severe. In order to avoid losing marks in your final assignment, or even failing, you must be careful to reference your sources correctly.

## What is a reference?

A reference is just a note in your assignment which says if you have referred to or been influenced by another source such as book, website or article. For example, if you use the internet to research a particular subject, and you want to include a specific piece of information from this website, you will need to reference it.

## Why should I reference?

Referencing is important in your work for the following reasons:

- It gives credit to the authors of any sources you have referred to or been influenced by.
- It supports the arguments you make in your assignments.
- It demonstrates the variety of sources you have used.
- It helps to prevent you losing marks, or failing, due to plagiarism.

## When should I use a reference?

You should use a reference when you:

- Quote directly from another source.
- Summarise or rephrase another piece of work.
- Include a specific statistic or fact from a source.

## How do I reference?

There are a number of different ways of referencing, and these often vary depending on what subject you are studying. The most important to thing is to be consistent. This means that you need to stick to the same system throughout your whole assignment. Here is a basic system of referencing that you can use, which consists of the following two parts:

1. A marker in your assignment: After you have used a reference in your assignment (you have read something and included it in your work as a quote, or re-written it your own words) you should mark this is in your text with a number, e.g. [1]. The next time you use a reference you should use the next number, e.g. [2].
2. Bibliography: This is just a list of the references you have used in your assignment. In the bibliography, you list your references by the numbers you have used, and include as much information as you have about the reference. The list below gives what should be included for different sources.

    a. Websites – Author (if possible), title of the web page, website address, [date you accessed it, in square brackets].

    E.g. Dan Snow, 'How did so many soldiers survive the trenches?', http://www.bbc.co.uk/guides/z3kgjxs#zg2dtfr [11 July 2014].

    b. Books – Author, date published, title of book (in italics), pages where the information came from.

    E.g. S. Dubner and S. Levitt, (2006) *Freakonomics*, 7-9.

    c. Articles – Author, 'title of the article' (with quotation marks), *where the article comes from* (newspaper, journal etc.), date of the article.

    E.g. Maev Kennedy, 'The lights to go out across the UK to mark First World War's centenary', Guardian, 10 July 2014.

# Appendix 3 – An example of scientific writing on cancer research

The following is the actual scientific paper reporting the experiment described here. You are not expected to understand the details of it! It is given only to show the format in which scientific reports are written.

*J. gen. Virol.* (1975), **27**, 127–134
*Printed in Great Britain*



# Transformation by a Temperature Sensitive Mutant of Rous Sarcoma Virus in the Absence of Serum

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(*Accepted* 6 *January* 1975)

SUMMARY

Cultures of chicken embryo fibroblasts infected with the temperature-sensitive transformation mutant of Rous sarcoma virus, *tsLA*24PR-A, were arrested between mitosis and S phase by exposure to serum-free medium at the non-permissive temperature (41 °C) for 2 days. On shifting to the permissive temperature (35 °C) the cells assumed a transformed morphology and increased uptake of [2-$^3$H]-deoxyglucose. There was a concomitant increase in acid insoluble [$^3$H]-thymidine uptake and the percentage of nuclei autoradiographically labelled with [$^3$H]-thymidine. This suggests that the virus transforming function can cause stationary cells to enter their growth cycle.

The level of release of infectious virus was shown to decrease on cell cycle arrest in serum-free medium and not to recover on a shift to 35 °C, when cellular DNA synthesis and transformation was induced. Cultures rendered stationary in medium containing serum depleted of multiplication stimulating factor did not show this reduction in virus production.

## INTRODUCTION

When Rous sarcoma virus (RSV) infects cells rendered stationary in serum-depleted medium the efficiency of cell transformation and virus replication is greatly reduced (Bader, 1966; Temin, 1967). However, under these conditions the virus becomes stably associated with the cell and when cellular DNA synthesis and division are stimulated by addition of serum, virus replicates and the cell transforms (Humphries & Temin, 1972). Since subsequent arrest and synchronization of transformed cultures is difficult, it is not yet clear to what extent events in the cell cycle are related to the continued expression of virus functions. We have obtained viable stationary cultures of RSV-infected cells by utilizing a transformation defective temperature sensitive (*ts*) virus mutant and arresting infected but phenotypically normal cells by serum depletion at non-permissive temperature. We show here that a late step in the control of transformation, represented by shifting the *ts* RSV-infected cells to permissive temperature, is serum independent. In contrast, production of infectious virus is greatly reduced in the absence of serum.

## METHODS

*Cell cultures.* Chicken embryo fibroblast (CEF) cultures of C/E phenotype were prepared from Brown Leghorn embryos (Houghton Poultry Research Station, Huntingdon, England)

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by standard techniques (Vogt, 1969). Cultures were maintained in Dulbecco's modified Eagle's medium supplemented with 10 % tryptose phosphate broth, 4 % calf serum and 1 % chick serum (complete medium: CM). Secondary cultures were seeded at $3 \times 10^6$ in 9 cm Nunc plastic Petri dishes and infected with $10^6$ focus forming units (f.f.u.) of the Rous sarcoma virus mutant *tsLA*24PR-A. This virus is temperature-sensitive in a function continuously required for the maintenance of cell transformation, the permissive temperature being 35 °C, the restrictive 41 °C (Wyke & Linial, 1973). It shows no defect in virus replication. As a further control for the effects of virus replication, duplicate cultures were infected with non-transforming Rous-associated virus 1 (RAV 1). After incubation for 3 days at 35 °C the infected cells were subcultured and transferred to 41 °C. Three days later they were passaged again and plated at $6 \times 10^5$ in 3 cm dishes for later measurement of [2-$^3$H]-deoxyglucose uptake, at $1{\cdot}5 \times 10^6$ in 5 cm dishes for measurement of [$^3$H]-thymidine uptake, virus release and cell counts, and at $2{\cdot}5 \times 10^5$ in 3 cm dishes for autoradiography. After 1 day they were washed twice in unsupplemented medium and maintained in Eagle's medium containing 10 % tryptose phosphate broth (ET) for a further 2 days at 41 °C. Cultures were then shifted to 35 °C (control cultures being maintained at 41 °C) with or without simultaneous replacement of ET by CM. Cell counts were made in a haemacytometer after removal of the cells with trypsin.

[2-$^3$*H*]-*deoxyglucose uptake*. This was based on the method of Weber (1973). Cultures were washed once in phosphate buffered saline (PBS) and incubated with [2-$^3$H]-deoxyglucose in PBS (0·2 mM; 1 $\mu$Ci/ml) for 9 min at 21 °C. After washing 4 times in ice-cold PBS and extraction with 2 ml 5 % trichloroacetic acid (TCA), 0·5 ml samples were counted in 10 ml Bray's scintillation fluid. The protein content of samples was determined by the method of Lowry *et al.* (1951) after solubilization in 1N-NaOH.

[$^3$*H*]-*thymidine uptake*. Cultures were incubated for 1 h with [$^3$H]-thymidine (1 $\mu$Ci/ml; Radiochemical Centre Amersham) in Eagle's medium at 35 °C, washed once in ice-cold PBS, twice in ice-cold methanol and allowed to air dry. Samples were solubilized in 0·7 ml of 0·2 N-NaOH, 0·5 ml of which was counted in 14 ml Bray's scintillation fluid after storing overnight at 4 °C.

*Autoradiography*. Cultures were incubated with [$^3$H]-thymidine (4 $\mu$Ci/ml) at 35 °C. After washing in PBS, TCA and methanol as for thymidine uptake, Kodak autoradiographic stripping film was attached to the dishes and exposed for 7 days. Dishes were then developed in D19 developer and cells were stained lightly in giemsa, and examined for labelled nuclei. In one experiment colchicine (Sigma Chemical Co.; 1 $\mu$g/ml) was added to the cultures when they were shifted to 35 °C.

*Release of infectious virus*. Virus was harvested by replacing the medium with 2 ml fresh ET or CM (supplemented with 1 % dimethyl sulphoxide), and collecting it 1 h later. Infectivity was determined by a standard focus assay (Vogt, 1969).

## RESULTS

### *Preparation of stationary cultures*

A two-day exposure of *LA*24-infected cultures to serum-free medium rendered the cells stationary, with incorporation of [$^3$H]-thymidine reduced to $< 10$ % of the level attained 8 h after subsequent serum stimulation. To find out in which phase of the cell cycle the stationary cells were blocked, cultures were treated at the time of shift from 41 to 35 °C with colchicine (1 $\mu$g/ml) to block cells at metaphase. After 7·5 h samples were labelled with [$^3$H]-thymidine (1 $\mu$Ci/ml) for 1 h and processed for autoradiography. The percentage of

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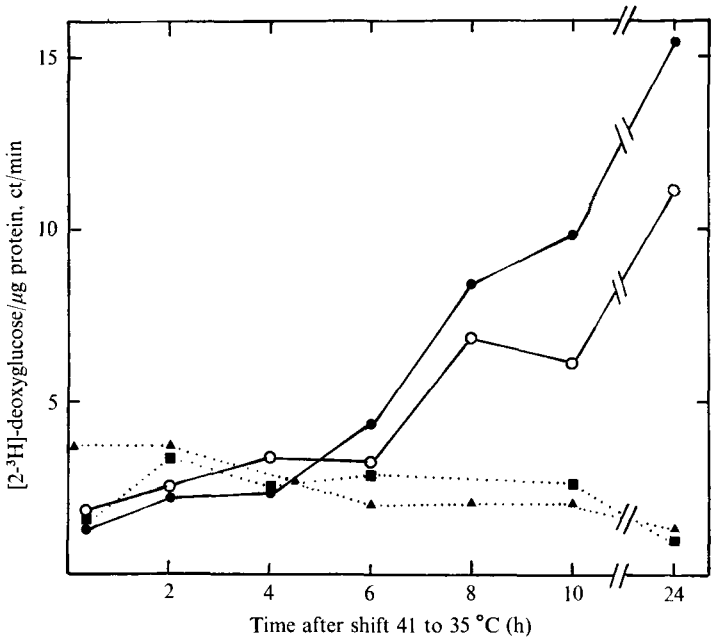


Fig. 1. Effect of temperature shift or addition of serum on uptake of [2-$^3$H]-deoxyglucose by cells made stationary in serum-free medium at 41 °C. ●—●, *LA*24-infected cells shifted from 41 to 35 °C. ○—○, *LA*24-infected cells shifted from 41 to 35 °C with medium containing serum added back at the time of shift. ▲ ..... ▲, RAV 1-infected cells shifted from 41 to 35 °C. ■ ..... ■, Non-stationary *LA*24-infected cells kept at 41 °C in medium containing serum.

labelled nuclei in cultures treated with colchicine (16 %) and in control cultures which did not receive the drug (14 %) was similar, showing cells incorporated [$^3$H]-thymidine without going through mitosis. This suggested that the cells had been stopped after mitosis and prior to the completion of DNA synthesis.

### *Uptake of* [2-$^3$*H*]-*deoxyglucose*

Ten hours after shifting such stationary cells from 41 to 35 °C the cell population, as viewed by low-power microscopy, had assumed a transformed morphology. Transformed cells have been shown to have a four- to tenfold increased uptake of [2-$^3$H]-deoxyglucose compared to normal cells and this can be used to monitor the appearance of transformation (Hatanaka & Hanafusa, 1970). The uptake of [2-$^3$H]-deoxyglucose by these cells increased soon after shifting down and by 24 h had increased fivefold to a level similar to that of fully transformed cultures kept at 35 °C throughout (Fig. 1).

### *Uptake of* [$^3$*H*]-*thymidine*

Uptake of [$^3$H]-thymidine showed a steady increase on shift down in the *ts*-infected cultures, while there was no increase in the low level of uptake in the serum-free cultures kept at 41 °C (Fig. 2). To establish whether the increased thymidine uptake was indicative of the initiation of DNA synthesis in the whole cell population, cells were incubated with 4 μCi/ml [$^3$H]-thymidine for 1 h at 2 h intervals and processed for autoradiography. From Fig. 3 it can be seen that the percentage of labelled nuclei shows a parallel increase to [$^3$H]-thymidine incorporation, suggesting the cells were in the S phase of the cell cycle.



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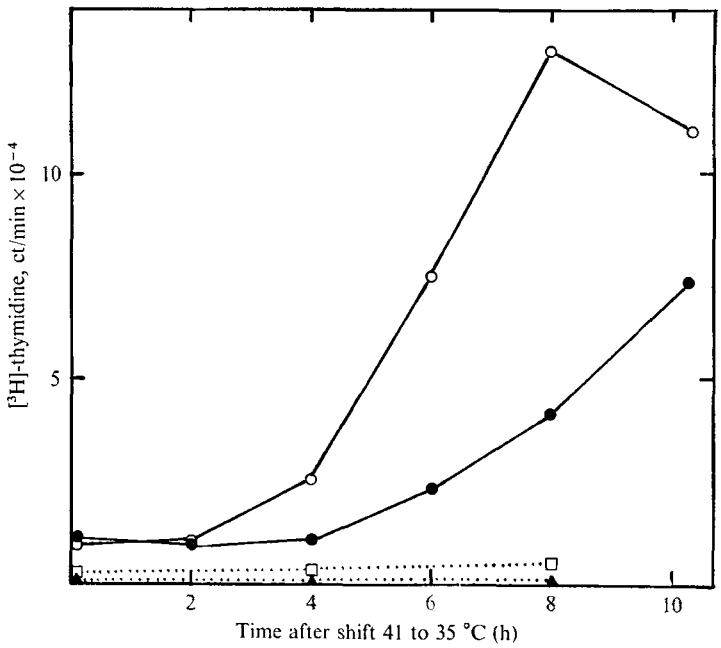


Fig. 2. Effect of temperature shift or addition of serum on uptake of [³H]-thymidine by cells made stationary in serum-free medium at 41 °C. ●—●, *LA*24-infected cells shifted from 41 to 35 °C. ○—○, *LA*24-infected cells shifted from 41 to 35 °C with medium containing serum added back at the time of shift. □ ..... □, *LA*24-infected cells kept at 41 °C in serum-free medium. ▲ ..... ▲, RAV 1-infected cells shifted from 41 to 35 °C.

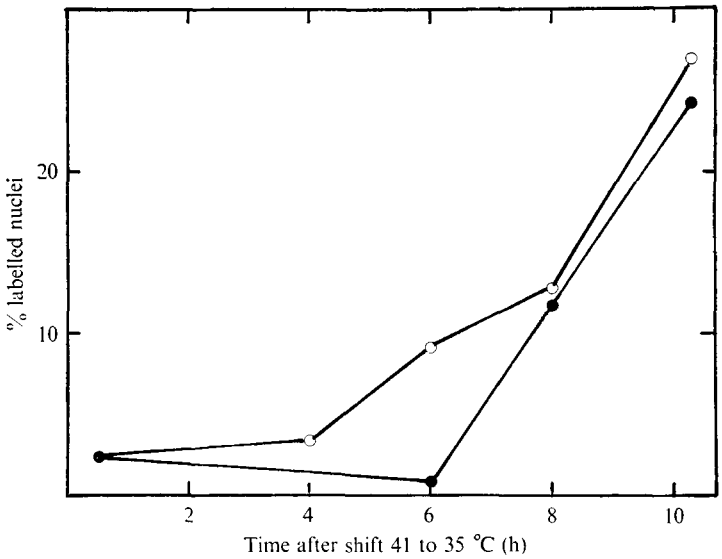


Fig. 3. Effect of temperature shift and addition of serum on nuclear incorporation of [³H]-thymidine pulses by cells made stationary in serum-free medium at 41 °C. Autoradiographs were prepared following a 1 h pulse of [³H]-thymidine at times after shifting stationary infected cells from 41 to 35 °C. ●—●, *LA*24-infected cells shifted from 41 to 35 °C. ○—○, *LA*24-infected cells shifted from 41 to 35 °C with serum added back at the time of shift.

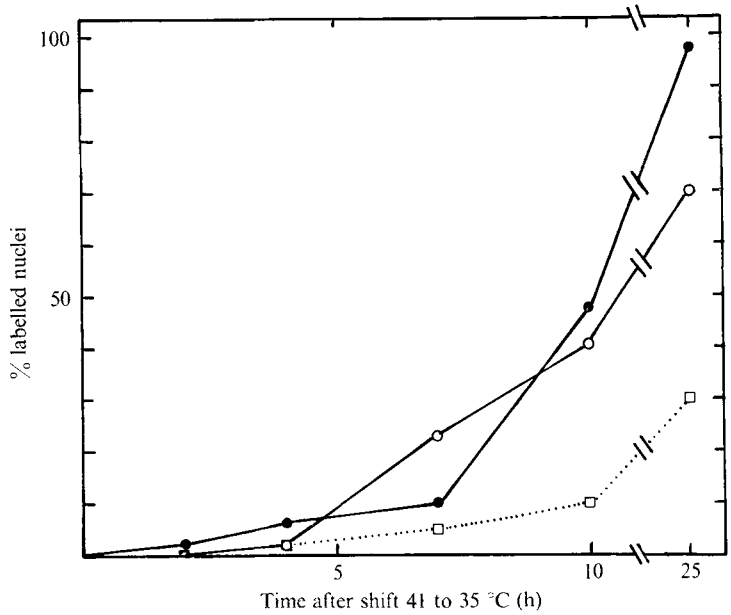


Fig. 4. Effect of temperature shift and addition of serum on accumulated nuclear incorporation of [$^3$H]-thymidine by cells made stationary in serum-free medium at 41 °C. [$^3$H]-thymidine was added to stationary *LA*24-infected cells at the time of shifting from 41 to 35 °C, the cells fixed, and autoradiographs prepared at the times shown. ●—●, *LA*24-infected cells shifted from 41 to 35 °C. ○—○, *LA*24-infected cells shifted from 41 to 35 °C with medium containing serum added back at the time of shift. □ ..... □, *LA*24-infected cells kept at 41 °C in serum-free medium.

Table 1. *Effect of temperature shift or addition of serum on cell numbers in* LA24 *infected cultures made stationary in serum-free medium at* 41 °*C*

| | No. of cells/dish |
|---|---|
| At time of shift from 41 to 35 °C | $2{\cdot}5 \times 10^6$ |
| 29 h later: | |
| Cells kept at 41 °C | $2{\cdot}8 \times 10^6$ |
| Cells shifted to 35 °C | $4 \times 10^6$ |
| Cells kept at 41 °C with medium containing serum added back at the time of shift | $5{\cdot}7 \times 10^6$ |
| Cells shifted to 35 °C with medium containing serum added back at time of shift | $5{\cdot}8 \times 10^6$ |

It is possible that the overall increase in DNA synthesis and sugar uptake observed in the cultures resulted from a minority population of cells which was not synchronized by exposure to the serum-free medium. To test this possibility, medium containing serum was added back to parallel cultures at the time of shift (Fig. 1 and 2). The level of sugar uptake and DNA synthesis reached in these cultures was approached by those starved of serum, indicating that a minority of the viable cells could not be responsible for the magnitude of the observed increases. When cultures were incubated continuously with [$^3$H]-thymidine for 25 h after shift and processed for autoradiography, the percentage of labelled nuclei, as seen in Fig. 4, was 70 to 100 %, indicating that most cells were viable. In keeping with these observations, Table 1 shows that cell numbers increased on addition of serum or on shifting cultures to 35 °C.



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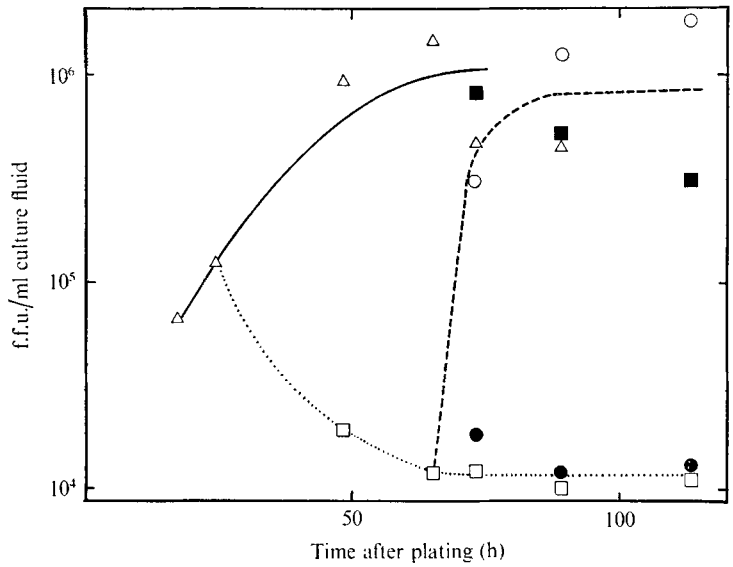


Fig. 5. Effect of temperature shift or addition of serum on production of infectious virus by cells made stationary in serum-free medium at 41 °C. △—△, Non-stationary cells kept in medium containing serum at 41 °C throughout. Cells changed to serum-free medium at 24 h and kept in serum-free medium throughout: □ ..... □, cells maintained at 41 °C; ● ..... ●, cells shifted to 35 °C at 65 h. Cells changed to serum-free medium at 24 h. Medium containing serum added at 65 h: ■ - - ■, cells maintained at 41 °C; ○- - -○, cells shifted to 35 °C at 65 h.

Table 2. *Effect of temperature shift or addition of serum on production of infectious virus by stationary* LA24 *infected cells.**

| | Virus titre (f.f.u./ml) | | |
|---|---|---|---|
| | 4 days after plating | 7 days after plating | 8 days after plating |
| Cells kept at 41 °C throughout, in depleted medium | $1{\cdot}2 \times 10^5$ | $1{\cdot}1 \times 10^5$ | $6{\cdot}3 \times 10^4$ |
| Cells shifted to 35 °C 7 days after plating and kept in depleted medium | — | — | $9{\cdot}5 \times 10^4$ |
| Cells kept at 41 °C; complete medium added 7 days after plating | — | — | $1{\cdot}1 \times 10^5$ |
| Cells shifted to 35 °C and complete medium added 7 days after plating | — | — | $1{\cdot}0 \times 10^5$ |

* Cells were made stationary through medium depletion according to the method of Humphries and Temin (1974).

*Production of infectious virus*

Virus release into the culture medium was measured throughout the course of the experiment in parallel cultures (Fig. 5) by harvesting the virus released into fresh medium (with or without serum) in 1 h. Cultures kept in serum-free medium showed a tenfold decrease in virus titres. Adding back medium containing serum at the time of shift restored this to the level found in cultures kept in serum throughout. The virus titre from cultures shifted to the permissive temperature showed a small increase consistent with the increased cell number, but remained much lower than that obtained with medium containing serum.

Since doing these experiments we have learned of the results of Humphries & Temin (1974), who synchronized *tsNY*68 SR-A-infected cells at the non-permissive temperature by

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growing them in 1·8 % foetal calf serum containing medium until it is depleted of multiplication stimulating factor. They found no reduction in virus titre after synchronization by this method, and re-addition of medium containing serum did not significantly change the titre of the virus produced. We have confirmed this observation in cells infected with *LA*24, and synchronized by their method (Table 2).

## DISCUSSION

Cells infected with the *ts*RSV mutant *LA*24 are phenotypically normal at 41 °C and can be arrested between mitosis and the S phase of the cell cycle by exposure to serum-depleted medium. On a shift to 35 °C these cells transform (Fig. 1), synthesize DNA (Fig. 2) and increase in cell number (Table 1). These data suggest that passage of the cells through their growth cycle in the absence of serum factors needed for the growth of untransformed cells is related to expression of the virus *ts* transforming function. This finding agrees with that of Renger & Basilico (1972), who used a cell line *ts* for expression of SV40 transformation but contrasts with that of Leong, Levison & Bishop (1972), who found that wild-type RSV-transformed cultures were rendered stationary by incubation in serum-free medium. A possible reason for this discrepancy is that the cultures of Leong *et al.* were probably blocked in $G_1$ by a lowering of the medium pH as a result of the higher cell numbers in the system they used. Transformation may be unable to overcome a block of this kind.

Two observations suggest that the induction of the virus-transforming function is the cause of the cells overcoming the cell cycle block. Firstly, temperature shift alone induces cells to incorporate thymidine to a level approaching that seen in cultures fed with medium containing serum. Secondly, the increase in thymidine incorporation in serum-free medium occurs reproducibly less rapidly in three experiments than the increase when serum containing medium was added at the time of shift (Fig. 2). The delay seen in the absence of serum may reflect a need for the *ts* virus function to operate before cells can proceed through their cycle. It will now be interesting to determine what effect inhibitors of various parameters of virus-induced cell transformation have on this cell cycle stimulation.

One aim of this study was to define methods of synchronizing mass cultures of RSV-transformed cells. Although transformation on shifting to 35 °C is satisfactory, the onset of cell DNA synthesis in the absence of serum appears relatively asynchronous, and a better hope for achieving synchrony lies in the addition of serum at the time of shift to permissive temperature. A delay is seen in stimulation of 2-deoxyglucose transport by serum addition compared to that observed by Sefton & Rubin (1971). This could be because our experiments used serum-free rather than serum-depleted medium to achieve the stationary cultures. When methods of synchrony have been perfected it will be possible to study the expression of transformed cell parameters at different stages of the cell cycle. The experiments reported here only permit the conclusion that the expression of the transformed phenotype may reappear in such cells prior to a complete round of DNA synthesis, a finding agreeing with the observation of Martin *et al.* (1971) that infected cells transform on shifting to 35 °C in the presence of an inhibitor of DNA synthesis. These observations do not eliminate the possibility that transformation may be affected by cellular factors which are produced at specific times in the cycle but which persist through one or more cycles. Synchronized cells which have been maintained in a stationary untransformed state long enough for such factors to decay are needed to investigate this. The use of different transformation defective *ts* mutants further broadens the scope of such an approach.

The finding that production of infectious virus is reduced in cells maintained in serum-free

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medium agrees with that of Leong *et al.* (1972). However, we have also confirmed with *LA*24 the observation of Humphries & Temin (1974) that virus replication is normal when cells are incubated in medium depleted of serum multiplication stimulating factor (Table 2). We conclude that a component in serum, distinct from the multiplication stimulating factor, is needed for normal virus production. When fresh medium containing serum is added to serum-free cultures the onset of virus production is rapid (Fig. 5), suggesting that replication is blocked at a late stage of maturation or release. The data of Leong *et al.* (1972) can be interpreted similarly. An alternative possibility, the failure of virus RNA transcription in serum-free cultures, seems less likely in view of this rapid appearance of virus and the fact that some virus RNA is presumably transcribed since the cells transform in the absence of serum. The possible blocks to virus production in the absence of serum should now be investigated more fully.

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# Notes

# Notes

# Can a single gene cause cancer?

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