CLONING AND STEM CELLS

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Important points

- Cloning is the creation of an exact genetic replica of a small segment of DNA, a cell or a whole organism. Identical twins are an example of human clones that are created naturally
- **DNA Cloning**, also known as recombinant DNA technology, molecular cloning and gene cloning, is used to produce many copies of a gene or DNA segment to be analysed for human genetic testing for medical, paternity and forensic purposes, the development of drugs and treatments such as pharmacogenetics and gene therapy
- **Reproductive Cloning,** also known as adult DNA cloning, is used to produce a genetic duplicate of an existing or previously existing organism e.g. Dolly the sheep in 1997 and many other animals since then including dogs and pigs.
  - Safety considerations and ethical issues in human cloning have led to universal condemnation and banning of the process: the concepts of parenthood, 'family' and views of social responsibilities are all challenged by human reproductive cloning
- **Therapeutic cloning to produce stems cells** is used to create undifferentiated cells capable of developing into most of the 220 types of cells found in the human body (eg. blood cells, muscle cells, nerve cells etc) for treatment of some conditions and the effects of trauma. Creating stem cells that contain the individual's own DNA would enable transplantation without the risk of tissue rejection
- The stem cells have to be able to be isolated from a source, grown in the laboratory, turned into the specific cell type needed for treatment and then trialled clinically to ensure that the new tissue or organ poses no risk to the patient. This area is still a matter of much research
  - When using cloned embryos as the source, stem cells are removed after the embryos have divided for about five days; the process destroys the embryo which is a major ethical consideration
  - An adult stem cell is an undifferentiated cell found among differentiated cells in a tissue or organ, can renew itself, and can differentiate to yield the major specialised cell types of the tissue or organ. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are located
- Australia has passed legislation banning human reproductive cloning (Prohibition Of Human Cloning Act 2002) but has treated therapeutic cloning differently by passing the Research Involving Human Embryos Act 2002 (and re-affirming this Act in 2007)

What is cloning?

Cloning is the creation of an exact genetic replica of a small segment of DNA, a cell or a whole organism.

- Identical twins are an example of human clones that are created naturally
- In contrast, Dolly, the cloned sheep, who was created artificially in a laboratory in Scotland in 1997

Dolly's creation led to international awareness of 'cloning' but there are several different types of cloning that can be used for purposes other than producing a genetic replica (or identical) twin of a whole organism like humans.

Types of cloning

(a) DNA Cloning

DNA cloning is also known as recombinant DNA technology, molecular cloning and gene cloning.

It is a form of cloning that has been around since the 1970s and the technique is common practice in molecular biology laboratories when multiple copies of genes need to be made for testing for health and forensic purposes (see Genetics Fact Sheets 21 & 22).

How DNA cloning is done

DNA cloning is used to produce many copies of a particular segment of DNA containing one or more genes, to be studied in the laboratory.

The DNA fragment of interest from an organism such as a human is incorporated into the 'plasmid DNA' of a bacterial cell. A plasmid is a circular self-replicating DNA molecule that is separate from the bacterial DNA (*Figure 26.1)*.

The plasmid containing the genes or DNA of interest is now a piece of 'recombinant DNA' made up of human and bacterial DNA. It is then put into a cell that will act as a host: as the host cell is copied over and over again, the recombinant DNA is copied as well. Bacteria are most often the host cells but yeast and mammalian cells can be used too. The end result is multiple identical copies of the same human DNA fragment or gene.

Benefits

Cloning is essential to enable enough copies of a gene or DNA segment to be analysed for human genetic testing to diagnose genetic conditions and enable predictive or presymptomatic genetic testing for genetic conditions in asymptomatic individuals or prenatally (see Genetics Fact Sheets 21 and 17C).

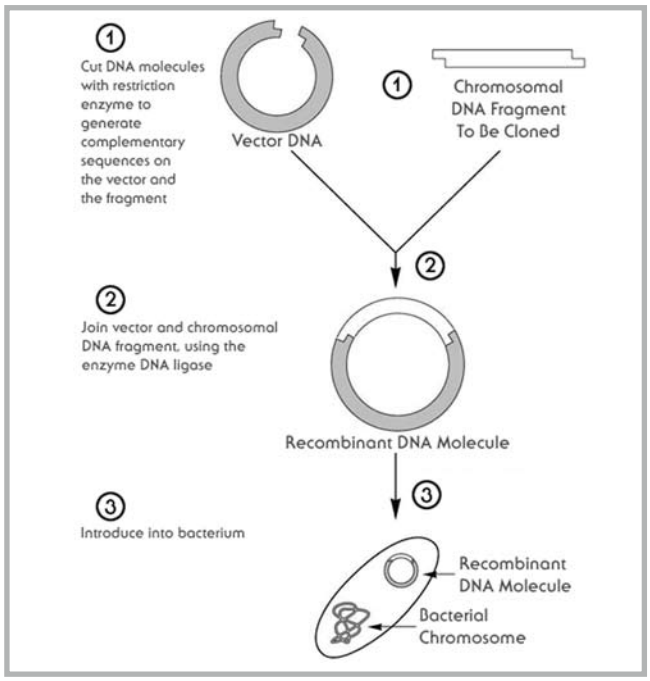

Figure 26.1: *By fragmenting DNA of any origin (human, animal, or plant) and inserting it in the DNA of rapidly reproducing foreign cells, billions of copies of a single gene or DNA segment can be produced in a very short time. The DNA fragment of interest from an organism such as a human is incorporated into the 'plasmid DNA' of a bacterial cell. A plasmid is a circular self-replicating DNA molecule that is separate from the bacterial DNA. When the recombinant plasmid is introduced into bacteria, the newly inserted segment will be replicated along with the rest of the plasmid. (source::://www.ornl.gov/sci/techresources/Human_Genome/elsi/cloning.shtml#whatis)*

In addition, forensic genetic testing relies on the ability to understand the information in the non-coding regions of the DNA ie. those areas that do not contain genes (see Genetics Fact Sheet 22).

Gene cloning is also important for the development of drugs and treatments such as pharmacogenetics (see Genetics Fact Sheet 25) and gene therapy (see Genetics Fact Sheet 27).

(b) Reproductive Cloning

Reproductive cloning is also called adult DNA cloning.

The purpose of this type of cloning is to produce a genetic duplicate of an existing or previously existing organism.

How cloning an adult animal is done

Dolly the cloned sheep produced in 1997 is an example of an animal produced by this kind of cloning as shown in *Figure 26.2*. She was produced in a process called ***somatic cell nuclear transfer*** **(SCNT)**. A somatic cell is any cell in the body other than the sperm or egg reproductive cells.

- In SCNT, the nucleus of a somatic cell isolated from the donor animal is put in an egg cell that has had its nucleus removed
- The egg cell is treated with an electric charge or chemicals to simulate fertilisation and cell division to produce an embryo
- The newly formed embryo can be implanted into a surrogate mother and carried to term. An embryo created by SCNT is a genetic replica of the donor animal

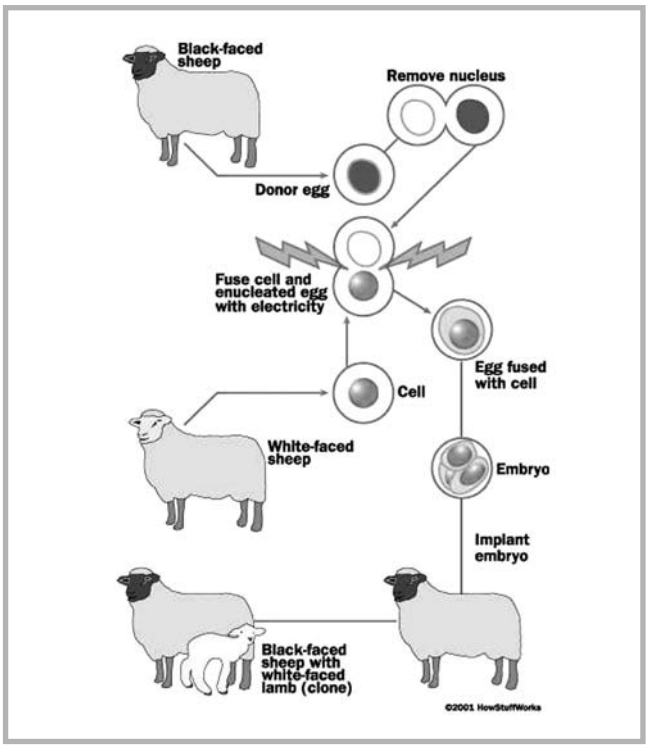

Figure 26.2: *Cloning of Dolly step by step. 1: Skin cells removed from the udder of a white faced Finn Dorset Ewe and placed in a growth culture containing very low nutrients: the cells are starved, stop dividing and the active genes are switched off. 2: The DNA is removed from an egg cell from a Scottish Blackface Ewe leaving the egg cell cytoplasm containing mitochondria. 3: The skin cell and the egg cell are placed next to each other and subjected to an electric pulse to cause fusion. The fused cell contains 46 chromosomes from the Finn Dorset Ewe in the cytoplasm of the Scottish Blackface Ewe. The fused cell is given a 2nd electric pulse to activate cell division and turn on the genes necessary for growth of the embryo. 4: The embryo is then implanted into the uterus of a different Scottish Blackface Ewe. Dolly, a cloned white faced Finn Dorset lamb, is born. (source of picture http://science.howstuffworks.com/cloning2.htm)*

An important step in the development of the cloning technology of whole animals is being able to activate the genes that are needed for an embryo to grow and develop that are inactivated soon after their job has been done. Adult cells therefore had to have these genes 'turned on' again.

The discovery of how to do this embryonic gene activation enabled cloning of Dolly and the technique has since been replicated with a number of other animals including mice, pigs, goats, cats, rabbits and cows.

It should, however, be noted that a clone such as Dolly produced by SCNT *is not an exact* replica of the donor animal. This is because while the white-faced lamb clone called Dolly has the same chromosomal DNA located in the nucleus as the white-faced donor sheep, the clone's mitochondrial DNA comes from the egg of another animal – in this case a black-faced sheep. The mitochondrion is an organelle found in the cytoplasm of cells that contains its own fragments of DNA and therefore its own genes (see Genetics Fact Sheet 12).

The difference between an embryo created by SCNT versus an embryo created naturally is where the two sets of chromosomes that make up the embryo came from.

- We all normally have 46 chromosomes in our body's somatic cells, 23 we receive from our mother via the egg and 23 from our father's sperm
- In SCNT, the egg cell's single set of 23 chromosomes contained in its nucleus is removed and replaced with a nucleus from a somatic cell from the donor that contains 46 chromosomes
- Thus, the cloned embryo receives all of the chromosomes from one adult somatic cell, rather than a mixture of genetic information from two parents

Benefits and limitations

Reproductive cloning can have many uses;

- If the low success rates and issues of safety could be improved as discussed below, the technology can be used to mass-produce animals with special qualities, such as animals that are important agriculturally or are able to produce helpful drugs for human use
- SCNT has environmental uses in that it can be used to repopulate endangered species, as has been shown with the wild ox and the gaur
- Some supporters of human reproductive cloning also see it as a way of overcoming male infertility, where other methods of assisted reproduction have failed (*Figure 26.3*)

The difficulties however in producing cloned animals are reflected in the number of abnormalities seen in the experiments done to date.

- For example, Dolly was the only survivor from 277 embryos cloned in the experiment conducted by Wilmut at the Roslyn Institute in Scotland. Dolly died in 2003 at the age of six. Prior to her death, Dolly had lung cancer and was arthritic. Although Dolly died much earlier than would be expected for a Finn Dorset sheep (from which she had been cloned) that usually live to be 11 or 12 years old, an examination of her cells at post-mortem showed no other abnormalities besides those associated with her cancer and arthritis
- At the same time that Dolly died, the first Australian cloned sheep, Matilda, died unexpectedly at a young age. She appeared healthy and energetic on the day she died, and the results from her autopsy failed to determine a cause of death

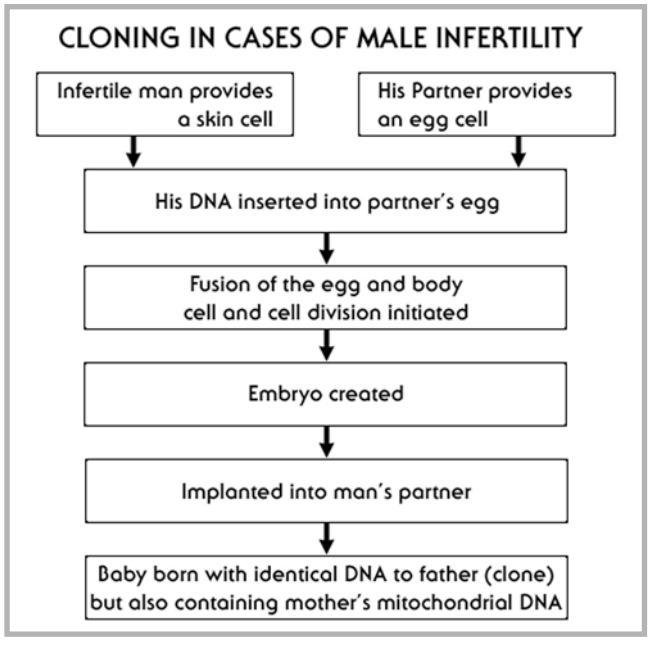

Figure 26.3: *Steps that need to be followed to clone a child if cloning technology is used where a man is infertile.*

Some animals are 'easier' to clone than others. Monkeys and chickens have proven difficult to clone and will require the developments of new techniques. The cloning of a horse in 2003 and a dog in 2006 is the result of overcoming some of these difficulties.

One of the reasons for the high rates of death, disability and abnormalities in cloned animals is perhaps in the reprogramming of the genetic make-up from an adult cell into an embryonic cell that must take place.

Some genes require to be passed to an embryo through the egg or sperm (***genetic imprinting*** –see Genetics Fact Sheet 15) for them to be active. Imprinted genes are involved in fetal growth and may explain why some cloned fetuses have 'overgrowth' syndromes.

In November 2001, scientists from Advanced Cell Technologies (ACT), a USA biotechnology company, announced that they had cloned the first human embryos for the purpose of advancing, therapeutic research. To do this, they collected eggs from women's ovaries and then removed the genetic material from these eggs with a very fine needle. A human adult skin cell was inserted inside the enucleated egg to serve as a new nucleus. The egg began to divide after it was stimulated with a chemical. This process was carried out with eight different eggs, of which only three began dividing, and only one was able to divide into six cells before the experiment was stopped.

Ethical considerations

In January 2003 a biotechnology group called Clonaid associated with the Raeilian movement, a religious sect, announced the birth of a cloned baby named 'Eve' but has never been confirmed independently. There was universal condemnation of the possibility of a cloned human.

Human reproductive cloning has raised many concerns, not the least of which are the safety considerations:

- More than 90% of offspring from cloning are not viable: only one or two offspring are viable for every 100 cloning attempts
- Cloned animals have higher rates of cancer and infection, and a range of other disabilities and conditions
- While the animal may seem healthy at birth, problems often have appeared later in the life of the resulting clone, so that mature clones have often undergone sudden, unforeseen and unexplainable deaths

While these technical difficulties may be overcome in the future and may enable cloning of animals with a greater success rate, the use of reproductive cloning of humans is viewed differently.

- Intellectual and emotional development is essential for human growth and health. It is also important to realise that a person is much more than a product of their genes

- If it were possible to produce a cloned human, the clone would not be a duplicate of that person, other than the nuclear DNA. As explained in Genetics Fact Sheet 1, a person is a product of their environment as well as their genetic make-up. Even identical twins have subtle differences between them
- The concepts of parenthood, 'family' and views of social responsibilities are all challenged by human reproductive cloning

(c) Therapeutic cloning to produce stems cells

The aim of therapeutic cloning is to harvest stem cells so the cells can be used to study human development and as treatments for some diseases. The purpose of this method of cloning is reflected in its name: to enable the correction of health problems.

The aim is to create stem cells containing the person's own DNA that could be grown in the laboratory and then transplanted into them without the risk of tissue rejection.

The stem cells are undifferentiated cells capable of developing into most of the 220 types of cells found in the human body (eg blood cells, muscle cells, nerve cells etc) that have different functions.

Diseases that might be treated by transplanting cells generated from human stem cells include Parkinson disease, diabetes, traumatic spinal cord injury, Duchenne muscular dystrophy, heart disease, and vision and hearing loss.

Using cloned embryos

When using embryos as the source of the stem cells, this type of cloning is also known as therapeutic cloning, biomedical cloning, embryo cloning or artificial embryo twinning.

This type of cloning essentially mimics the natural process of producing identical twins or triplets.

- Identical twins are formed when the fertilised egg tries to divide into a two-cell stage but the two cells separate instead
- The two separate cells continue dividing on their own and eventually develop into two genetically identical individuals

How embryonic cloning is done

In embryonic cloning, cells are removed from an embryo after it has divided for about 5 days. At this stage, the embryo is called a *blastocyst*. When the cells are removed the embryo is destroyed.

The cells of an embryo are an excellent source of stem cells because they have not yet been differentiated into the cells of specific tissues or organs of the body. While there are around 20,000 genes in each body cell, only those genes that are needed for the function of the cells of the tissue or organ are 'turned on'. The remainder are inactive. For example, brain cells will have different genes sending instructions to the cells than liver cells. Embryonic stem cells still have all the genes needed for the function of the body's cells to be able to be 'turned on' and so can be differentiated into any type of body cell.

To generate cultures of specific types of differentiated cells — heart muscle cells, blood cells, or nerve cells, for example — scientists try to control the differentiation of embryonic stem cells. They change the chemical composition of the culture medium, alter the surface of the culture dish, or modify the cells by inserting specific genes. Through years of experimentation scientists have established some basic protocols or 'recipes' for the directed differentiation of embryonic stem cells into some specific cell types.

If scientists can reliably direct the differentiation of embryonic stem cells into specific cell types, they may be able to use the resulting, differentiated cells to treat certain diseases at some point in the future.

Using adult cells

An alternative source of stem cells extracted from embryos for therapeutic cloning are adult stem cells that can be extracted from adult tissue such as the bone marrow without harm to the individual.

- An adult stem cell is an undifferentiated cell found among differentiated cells in a tissue or organ, can renew itself, and can differentiate to yield the major specialised cell types of the tissue or organ
- The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are located
- Somatic stem cell is an alternative term instead of adult stem cell
- Unlike embryonic stem cells, which are defined by their origin (the inner cell mass of the blastocyst), the origin of adult stem cells in mature tissues is unknown.

Adult stem cells occur in many more tissues than was once thought possible, and adult stem cells from bone marrow that form into mature blood cells have been used in transplants for 30 years. Tissues include brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin and liver.

There are, however, often only a very small number of stem cells in each tissue. Stem cells are thought to reside in a specific area of each tissue where they may remain quiescent (non-

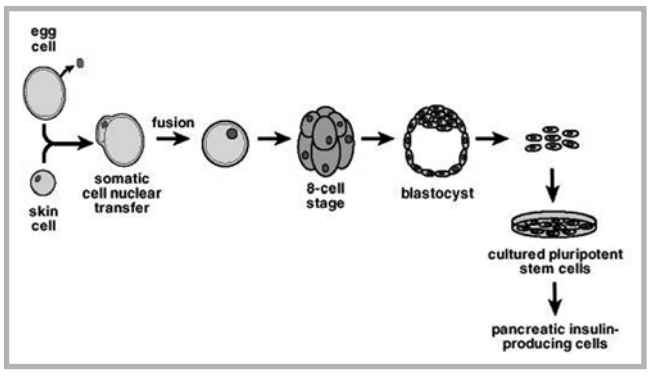

Figure 26.4: *A doctor takes a sample of skin cells from the patient and isolates their DNA. Next, a donor egg cell, emptied of its own genetic contents, is injected with the DNA from the patient. The embryo is nurtured to grow and divide into a blastocyst. Some blastocyst cells are harvested and coaxed with growth factors to mature into insulin-producing cells. Finally, millions of insulin-producing cells are injected back into the patient. In an ideal world, the patient's diabetes is temporarily 'reversed', with no side effects. (Picture: adapted from Stem Cells: A Primer, US National Institutes of Health). Source: Cloning around with stem cells http://abc.net.au/science/slab/stemcells/default.htm).*

dividing) for many years until they are activated by disease or tissue injury.

Certain kinds of adult stem cells seem to have the ability to differentiate into a number of different cell types, given the right conditions. If this differentiation of adult stem cells can be controlled in the laboratory, these cells may become the basis of therapies for many serious common diseases.

Figure 26.4 is an example of the way in which therapeutic cloning using adult stem cells could be used in the treatment of diabetes type 1 where the damaged pancreatic cells that produce insulin are replaced, removing the requirement for daily administered insulin (see Genetics Fact Sheet 57).

Benefits and limitations

Whatever their source, before stem cells can be used to treat health problems, a number of barriers have to be overcome.

1. The stem cells have to be able to be isolated from a source and then grown in the laboratory
2. The stem cells have to be able to be turned into the specific cell type needed for treatment

These first two hurdles have been passed for most of the 220 cell types in the human body.

Overcoming the next barriers of applying the technology clinically and ensuring that the new tissue or organ poses no risk to the patient is still a matter of much research.

While one organ, the skin, is already able to be grown in the laboratory to create a self-compatible skin graft, skin is relatively easy to grow because the mature, differentiated skin cells are still able to divide and produce more cells to repair damage. The cells of other organs or tissues do not have this ability.

An alternative is to create genetically modified pigs from which organs suitable for human transplantation could be harvested. Pig tissues and organs are the most similar to humans of the animal species that have been cloned. The transplant of organs and tissues from animals to humans is called *xenotransplantation*. One of the major concerns, however, in the transplant of pig tissues and organs to humans, is the transmission of pig viruses to humans.

Stem cells have the potential to be used to replace faulty cells in people affected with cancer, cardiac disease, diabetes type 1 and degenerative conditions such as Parkinson disease and Alzheimer disease. For example, recent work with stem cells inserted into damaged heart muscle has led to the regeneration of cells. Other work in mice has shown the potential to use stem cells to produce pancreatic cells for the production of insulin for the reversal of diabetes type 1.

While adult stem cells may overcome some of the ethical considerations (see below), further studies are needed to determine their efficacy in differentiating into a variety of new types of cells.

Initial work suggested that their potential may be limited but recent work has suggested that this may be wider than first indicated.

Ethical considerations

The therapeutic cloning technology used to harvest stem cells ultimately results in the destruction of an embryo. The creation of embryos specifically for the purpose of destroying them causes concern for some people.

The advantage of using adult cells as a source of stem cells is that it would avoid this ethical issue of destruction of the embryo.

Currently, embryos that are used for the production of stem cells are those created by infertile couples during *in vitro* fertilisation (IVF) programs. Usually more embryos than are needed to have a baby are produced. Some couples have agreed for their excess embryos to be used for stem cell research.

Policies

Cloning presents many complex ethical questions. A few to consider are who should have access to the technologies, how will the use of the technologies be monitored, who gets to decide what aspects of cloning are morally acceptable and legal to use and which are not. So even if it is possible to clone humans – should we?

In 1999, the Council of the Australian Academy of Science unanimously endorsed a position statement called 'On Human Cloning' (http://www.science.org.au/nova/043/043box03.htm) that recommended a ban on human reproductive cloning but recognised the potential of therapeutic cloning and considered that it should be fostered in research.

Australia has passed legislation banning human reproductive cloning (Prohibition Of Human Cloning Act 2002) but has treated therapeutic cloning differently by passing the Research Involving Human Embryos Act 2002.

- The object of this Act was to address concerns, including ethical concerns, about scientific developments in relation to human reproduction and the utilisation of human embryos by regulating activities that involve the use of certain human embryos created by assisted reproductive technology(ART)
- At the time of passing the Act, a three-year moratorium was placed on the use of excess embryos for research. In 2007, however, approval was given to use embryos (created after April 5 2002 by assisted reproductive technologies with the aim of achieving a pregnancy) that are in excess of a couple's needs, and with their informed consent, for stem cell research. This approval applies when that research is governed and overseen by a committee of the National Health and Medical Research Committee (NHMRC)
- This Act was reviewed in 2004 pending its repeal in April 2005 but in 2007 an Act was passed to continue to allow scientists to create embryos through therapeutic cloning and extract their stem cells for use in medical research

Currently, therapeutic cloning research is being conducted in private laboratories in the USA and in both government and private laboratories in the UK, Japan, France, Australia, and other countries.

Other Genetics Fact Sheets referred to in this Fact Sheet: 1, 12, 15, 17C, 21, 22, 25, 27, 57

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