

Iron regulatory protein Research Paper Sample - MLA

Sample Research Paper

Iron regulatory protein

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Characterization of the human iron regulatory protein 1 (IRP1)

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Abstract

The primer design is specified in the strata gene protocol in which the oligonucleotides need to be put through polyacrylamide gel electrophoresis (PAGE) (Zheng et al, 45-43). In order to design the primers, one will need the specifications below in the measurements given. The procedure then proceeds to the next step which involves amplification of the DNA template contained in the reaction mix. Once the process of template DNA amplification is over, the procedure for IRP1 identification and characterization goes to the gel phase. Gel phase is followed by visualization and there should be a band corresponding to your polymerase chain reaction product (Zheng et al, 45-12). When the band is a strong primer dimer band can demonstrate that the primer-primer annealing is favored. After visualization, there is digestion after which the last reaction is transformed into competent cells. This is followed by making the sequence into minipreps. The minipreps are used to check for any changes that have lead to mutations. The process is carried out after which the sample is put in a centrifuge. Protein lysates are afterwards allowed to resolve on SDS polyacrylamide gel electrophoresis. Then it is transferred to nitrocellulose membrane. It is allowed to set for an entire night. After setting for the whole night the sample is incubated. During incubation, an affinity rabbit serum that corresponds to the human compound is used (David, 34-57). The rabbit serum is placed against the human IRP and ammonium terminal peptide. The sample is also taken through northern blotting for ribo nucleic acid analysis, Spectrophotometry. This occurs using bio Rad protein assay to analyze the sample by determining its charge and molecular weight (David, 34-57).

Summary of experiments and results expected

The reaction mix for the experiments will contain the following reagents and compounds. The different proportions for the reaction and the mixtures are also indicated in the steps which are summarized below. 0.5 micro litres of forward primer (forward primer has the bases arranged from the three prime end to the five prime end) which is 2.5 p moles per micro litre, 0.5 micro litre reverse primer; (the reverse primer is different from the forward primer (“the difference is directional”) .The reverse primer unlike the forward primer had the nucleic acid bases moving in the opposite direction (Zheng et al, 45-12).The reverse primer therefore only moved from five prime regions to three prime regions.) 0.25 micro litre 40mM of di nucleotide tri phosphates mixtures, 1.25 micro litres times 10 pfu ultra buffer which contains magnesium, 1 micro litre of the DNA template to be used, this deoxyribonucleic acid template is known as the parent template. This is because, just like a real parent it forms the basis for the production of the other primers.0.25 pfu ultra hot start strata gene and finally you measure 8.75 micro litres of sterile water (Zheng et al, 45-12).This sterile water is available commercially and is usually bought as PCR water for polymerase chain reaction.

The total mixture for this reaction will constitute of 12.5 micro litres of all the substrates and specimens. The next process involves suspending the cells 0.2M sucrose, then suspending it in 100ml hepes at a pH of 7.4 then treat with 0.007 % digitonin. This process is carried out after which the sample is put in a centrifuge. The process that takes place in the centrifuge occurs at 4° C. the centrifuge is set to rotate making 1800 spins per minute for every gram in the measurement of the sample. This

entire process is allowed to take place for five minutes. The supernatants are then allowed to spin in the centrifuge. This time the centrifuge is set to make 150000 rotations per minute and the procedure is allowed to go on until a total of 20 minutes. The supernatants collected are then stored at 80° C. these now constitutes the cytosolic extracts. Then it is transferred to nitrocellulose membrane. This is allowed to set for an entire night. After setting for the whole night the sample is incubated. During incubation an affinity rabbit serum that corresponds to the human compound is used. This rabbit serum is placed against the human IRP and ammonium terminal peptide (Dycke et. al, 22-28).

Literature Review

Iron regulatory proteins (IRPs) are proteins in the body that control the balance of iron there by bringing about iron homeostasis in the human body. Iron homeostasis is the state in which the balance of irons in the body is just right for normal physiological functioning (David, 34-57). Any measures above the normal physiological measurements are hyper normal and can bring about deficiencies. The above normal levels are most common causes of diseases (Rouault, 67-43). In this care we have iron deficiency diseases whose symptoms include reduction in blood and oxygen supply to the needy tissues. Some iron deficiencies include iron deficiency anemia and zebra anemia. Iron is important in the human body for formation of red blood cells among many other functions (Dycke et. al, 20-31).

The main function of red blood cells being the formation of red blood cells brings about the interrelationship iron has with a disease like anemia, especially iron deficiency

anemia and zebra anemia. The deficiencies in iron form the common results of lack of homeostatic balance of the iron compound in the body (David, 34-57).

These deficiencies can be genetic where by the affected individual had the condition passed down to them from one parent or both parents. The iron deficiency diseases can also be because of nutritional factors. The lack of iron rich foods in the diet will automatically lead to iron deficiency in the body (Rouault, 67-43). This is because the main supply of iron rich compounds in the body is the iron rich foods that form part of the meal. When they are not supplied with sufficient iron, these meals will cause an individual to suffer from iron deficient anemia that has a nutritional basis (David, 34-57). Another possible cause of iron deficiency in the body is extreme loss of blood from the body. This extreme loss of blood can be caused when a person is involved in an accident or experiences severe burns that lead to loss of water in the body. The water is lost with the iron component in the heme part of a blood compound known as hemoglobin (Dycke et. al, 23-32).

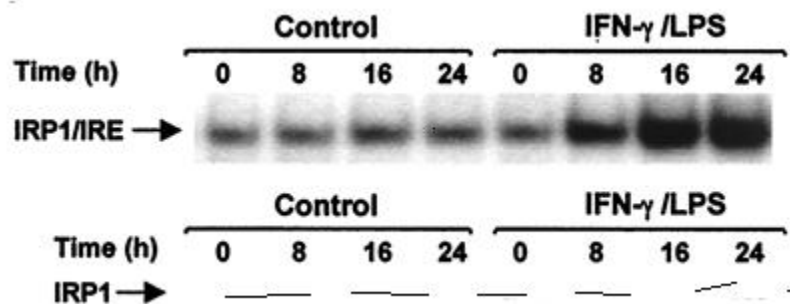
Iron regulatory proteins (IRPs) are specifically cytosolic Ribonucleic acid (RNA) binding protein that will in a specific manner manage messenger RNA (mRNA) for storage, movement and use of iron mineral after the transcription process is over (Dycke et. al, 23-31). The IRPs are ubiquitine in nature and serve to therefore control iron related processes in the body (Rouault,67-43).

During the regulation of the processes in the cell, Iron Regulatory proteins manage the storage of iron which is stored in the body. Iron in the body is stored in the form of a ferritin compound (David, 34-57). The ferritin compound is a less toxic form in

which the normal iron component is stored in the body without resulting into any physiologic imbalances (Dycke et. al, 21-27). There is also regulation of the movement of iron compounds in the body. This involves the transportation of iron from the source which is in the diet to its final destination. The most common source of iron in the body is the diet rich in iron compounds. This can be iron rich meals like mineral salts, beef and dairy products (David, 34-57).

Another source of iron in the body which eventually gets regulated by the human iron regulatory protein is contained in mineral supplements. These are given to people who have extreme iron deficiency in the form of drugs which are given as pills. In very extreme conditions of deficiency they can be given in dissolved states directly into the body system (David, 34-57). The transportation of iron in the body occurs by the binding of the iron compounds on various receptors in the body system. Once bound by using the lock and key hypothesis where by the receptors are very specific to their targets, the iron gets moved around the body (Dycke et. al, 22-29).

When iron reaches its final destination it is then that it is involved in its function in the human body, the function being the formation of the heme compound in the red blood cells component of the blood (Rouault, 67-43). Transport of iron in the body occurs by transport receptors which bind and transport iron compound through the body to where the iron compound is utilized under the regulation of human iron regulatory protein 1(Dycke et. al, 24-39)

Figure 1: IRP1 Expression (RAW 264.7 Macrophage Stimulated by IFN- γ and LPS)

Source: Oliveira, Leonor & Jean Claude Drapier. "Down Regulation of Iron Regulatory Protein 1". University of California, Los Angeles, CA. 2000.

Human Iron regulatory protein 1 also facilitates the process of iron utilization in the body once the iron compound reaches its final destination from the source where it is used up in the body. This is basically for erythroid alpha – amino/aevulinic acid synthesis. (Dycke et. al, 26-27). To serve their purpose in the human body, iron regulatory proteins bind to iron responsive elements (IREs) to ribonucleic acid stem loop structures in the section of messenger ribonucleic acid that do not translate called un translated region (UTR) . This occurs in the three prime to the five prime un translated region (Dyke et. al, 25). This binding forms a human iron regulatory protein 1 and iron responsive elements complex. The human iron regulatory protein 1 and iron responsive elements complex is very strong forming the stability of mRNA that is involved in human iron protein regulation in the human body (Dycke et. al, 27-29).

When there is less iron in the body, iron regulatory protein 1 binds to a complex compound (4Fe- 4s) which is cluster like compounds and they work in the body to

regulate cytosolic acotinase. This process of binding can bring about stability of mRNA. It can also cause prevention of the process of translation according to the coding sequence in question. This process of mRNA inhibition depends on the type of cell in question. Depending on the cellular conditions and body requirement at specific times, the level of inhibition will vary so that a cell can have more of the protein during specific physiological conditions or at certain times only. The inhibition of messenger ribonucleic acid reception occurs when the cellular physiologic requirements demands changes (Michael, 88-74). The body might be in need of certain types of proteins at specific times causing the regulation to be increased. The reverse happens when the body no longer needs this iron regulatory protein causing the down regulation of the production of the messenger ribo nucleic acid responsible for the production of human iron regulatory protein 1. Iron regulatory protein 1 has two functions and activities. This makes it have a dual purpose in the body, and also brings out the dual functioning of iron regulatory protein one (Michael, 88-74 & David, 34-57).

In one instance, the iron regulatory protein one serves the function of cellular respiration whereby it produces energy for cellular activities and functions in the form of ATP. This is the respiratory function of iron regulatory proteins when they are in the form of acotinase. The respiratory function of iron regulatory protein as acotinase takes place within the tri carboxylic acid cycle. The other form which is acotinase cytosolic form serves a totally different function. The cytosolic acotinase form serves the function related to iron regulatory protein 1 specifically (Michael, 88-74). The dual activities of iron regulatory protein 1 are thus balanced by cytosolic acotinase and are not controlled by the amount of iron present in the body. (Dycke et. al, 23-27).

Careful observation of iron regulatory proteins will reveal a double crystal structure. This is the form of structure which the crystal acotinase form of iron regulatory protein 1 will take. Acotinase is an enzyme protein. This enzyme acotinase serves its function in the tri carboxylic acid cycle also called the Krebs cycle that takes place in the mitochondria during the process of cellular respiration to produce energy for the cell in the form of ATP which stands for adenosine triphosphate. This adenosine tri phosphate is a constituent compound of adenine and three phosphate groups (Dycke et. al, 23-26). The cytosolic form of this enzyme acotinase has another function of carrying out the function of human iron regulatory protein 1. This compound has a hydroxyl section and a specific atom from the citrate compound. When they are substituted in a geometrically balanced manner, the two will come out as distinct crystal structures (Zheng et al, 45-12). This brings out the dual functioning of iron regulatory protein one. In this case there is one instance where the iron regulatory protein one serves the function of cellular respiration whereby it produces energy for cellular activities and functions in the form of ATP (Rouault, 67-43). This is the respiratory function of iron regulatory proteins when they are in the form of acotinase. Respiratory function of iron regulatory protein as acotinase takes place within the tri carboxylic acid cycle (David, 34-57). Other form which is acotinase cytosolic form serves a totally different function. This forms the second part of the dual function of the human iron regulatory protein 1. The cytosolic acotinase form serves the function related to iron regulatory protein 1 specifically. This is because it is the cytosolic form of acotinase crystal compound that make up the iron regulatory protein 1 (Zheng et al, 45-12).

For the maximization of the dual activity during the scarcity of iron compound in the body, for example in the cases of iron deficiency in the body, IRP1 binds 4Fe- 4s clusters and functions in the regulatory process (Zheng et al, 45-12). Human iron regulatory protein two on the other hand is different from the reaction of the human iron regulatory protein one. Unlike the human iron regulatory protein one, the human iron regulatory protein two will get degraded where there is insufficient iron in the body, for example in the cases of iron deficiency.

Zebra anemia is a medical condition in which the availability of oxygen for tissues is minimal (Michael, 88-74). This is because of a deficiency in the red blood cells to carry maximum oxygen from the lungs to the entire body and supply the body tissues. The main factor causing this condition is the regulation of the human iron regulatory protein 1 (IRP)- IRE complex. This occurs during the prolonged activation of iron regulatory protein 1. It causes change of protophoryn to transferrin bound presence of iron in the red blood cells formation tissues. (Dycke et. al., 25-29)

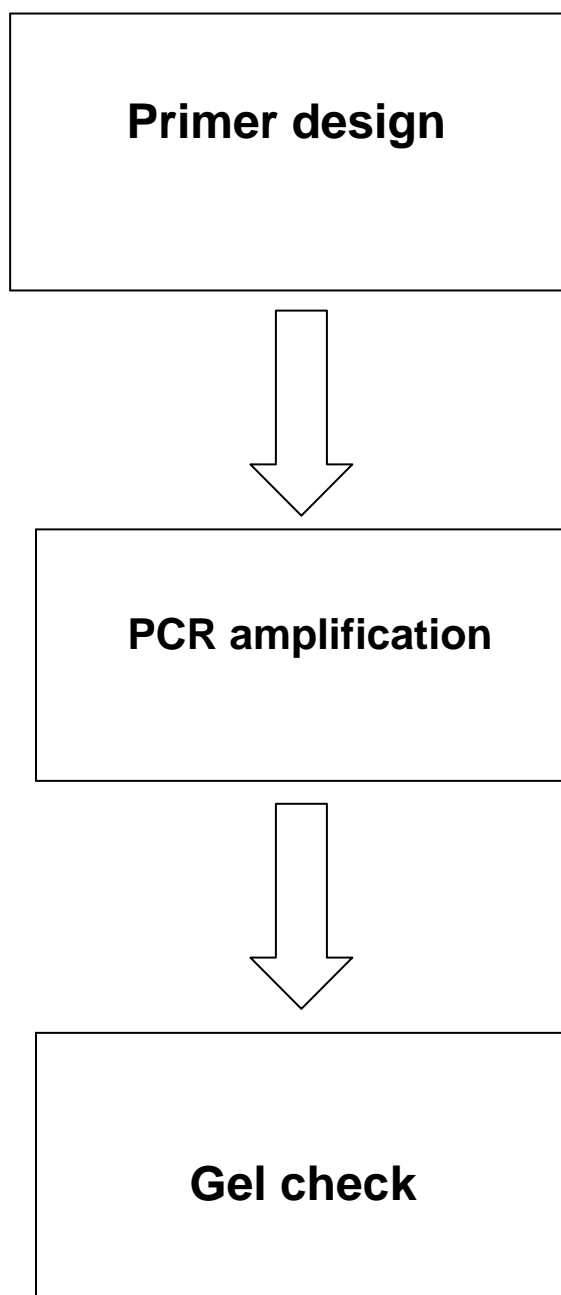
In short, Iron regulatory proteins (IRPs) control the translation of proteins involved in iron uptake which occurs after an intake of iron mineral in the meal. The iron regulatory proteins then go ahead to take part in the regulation of iron storage in the human body. Storage of iron is in the form of an iron compound called ferritin (Michael, 88-74). After storage the iron has to be moved through the body to the source where it is utilized. This occurs by use of binding factors. Iron is transferred from its source to the place of utility by use of binding factors. These are found in sites of reception where the iron receptors bind the iron compounds and transport them to the final destination

where they are utilized. Iron rich compounds in the body are mainly utilized in the formation of red blood cells (David, 34-57). This is specifically the formation of the heme compound found in the hemoglobin part of the red blood cells. This process occurs in the final stage of iron movement in the blood. The use of iron in the body is through the compound formation of erythroid alpha- amino/ aevulinic acid synthesis (Zheng et al, 45-12). The procedure for the characterization of the iron regulatory proteins starts with the formation of primers called primer design. These primers called oligonucleotides will be used in the polymerase chain reaction procedure that entails amplification of the parents strand (Michael, 88-74).

Hence the step that follows is the polymerase chain reaction procedure that amplifies the small parents' template into a substrate that can easily be observed and analyzed. The polymerase chain reaction step was followed by the gel electrophoresis step. In the gel electrophoresis step, the main gel that is used is the agarose gel for analysis of the polymerase chain reaction products (Michael, 88-74).

Materials and methods

Figure 2- Flow chart of the experiment



Procedure

The structural data that is used in the design of primers to be used are obtained from the protein databank. In order to access the protein data bank, one would need to have electrical and software utilities (David, 34-47). These will include the hardware part of information technology which is available and computers and other data processing machines and electronic devices. In this case the electronic device used is the computer (New England Biolabs, 21-29). The computers have to be in good working conditions with supply of power. Power is supplied in the form of electricity to make the functioning of the computer hardware possible and without any inconveniences. One will also be required to have some software utilities. Soft wares are programs that are installed on computers to carry out specific tasks while in the computer. The software can also be application packages that are programmed to carry out specific functions of the computer. These software will include specific programs like web browsers and software programs for FTP access (David, 34-47).

Once you have obtained the necessary utilities that are in the form of hardware and software programs, the next step is to select the files that will be used to access the soft wares for the primer design. The files can be accessed using searching methods like quick search, or RCSB (David, 34-47). Another way of access is the use of downloading method where the site containing the sequence is typed in the web browser and allowed to access the desired sequence. The address of the site containing the sequence is typed in the address panel of the web browser and the computer is allowed to locate the sequence (New England Biolabs, 21-29). These

sequences are stored in various databanks for sequences that can be accessed over the internet. The sources of sequences are contained in various data banks. These data banks can be for nucleic acid sequences (David, 34-47). The nucleic acid sequences include the RNA sequences and the DNA sequences. The sequences can also be those for protein sequences that are stored in specific data banks accessible over the internet. By combining the efforts of computer hardware, computer software and the human resource skills to access the protein, DNA and RNA sequences found in these data storage locations called data banks. The data banks which are change regularly are maintained by various groups. The groups in this case include mostly institutions. These can be universities or research centers and schools (Rouault, 67-43).

The primer design is specified in the strata gene protocol in which the oligonucleotides need to be put through polyacrylamide gel electrophoresis (PAGE). (Rouault, 67-43). In order to design the primers, one will need the specifications below in the measurements given ((Pantopoulos, *Iron Regulating Protein*).

0.5 micro litres of forward primer (forward primer has the bases arranged from the three prime end to the five prime end) which is 2.5 p moles per micro litre, 0.5 micro litre reverse primer; (the reverse primer is different from the forward primer, that is to say, "the difference is directional". The reverse primer unlike the forward primer had the nucleic acid bases moving in the opposite direction (David, 34-47). The reverse primer therefore only moved from five prime regions to three prime regions.) 0.25 micro litre 40mM of di nucleotide tri phosphates mixtures, 1.25 micro litres times 10 pfu ultra buffer which contains magnesium, 1 micro litre of the DNA template to be used, this

deoxyribonucleic acid template is known as the parent template. This is because, just like a real parent it forms the basis for the production of the other primers. 0.25 pfu ultra hot start strata gene and finally you measure 8.75 micro litres of sterile water. (Zheng et. al, 45-12). The procedure goes to the next step (David, 34-47). This step involves the amplification of the amplification of the DNA template contained in the reaction mix. Amplification occurs by use of polymerase chain (PCR) reaction which is use of a PCR machine to amplify nucleic acid samples. Polymerase chain reaction has three major steps. These steps are repeated and regulated to vary the results and output in different conditions (New England Biolabs, 21-29).

The three major steps that get regulated include the denaturing stage, annealing and extension step. The denaturing step involves extremely high temperatures using temperature resistant enzymes. This in most cases is the taq polymerase which can survive extreme temperatures. This removes the necessity to change the enzyme every time it gets denatured. This is because enzymes are like all enzymes which cannot withstand temperatures above forty degrees centigrade's (David, 34-47). During the step of annealing temperatures are allowed to drop this will allow the complimentary strands to anneal and form two new strand from the denatured double strand that was separated into tow strands (New England Biolabs, 21-29). After the annealed primers have reached their saturation, they are put at constant temperatures and allowed to extend. During the extension stage, the template primer is allowed to increase in size leading to the bulk production of the sample to from very little parent template. The Polymerase chain reaction is a step by step procedure that involves alteration of temperatures to make conditions suitable for each step (Zheng et. al, 45-12).

The Polymerase chain reaction procedure occurs with varying temperature changes. The procedure starts with heating the reaction mixture up to 95 °C which is done for up to 5 minutes (Michael, 88-74). After raising the temperatures, the template DNA sample will be denatured and the strands will be allowed to separate. This is called the denaturing step in the procedure where the template DNA strands get separated. The procedure then moves to the second step. This is the annealing step where the complementary DNA strands are allowed to join. This is done to target specific primers on the two separate strands (David, 34-47). The second step in Polymerase chain reaction of annealing occurs in three instances. The first instance involved lowering the temperatures to 60° C after which the Polymerase chain reaction is allowed to continue for up to 50 spins. The temperature is slightly raised to 68° C to allow further annealing (New England Biolabs, 21-29). This also goes on for a maximum of 50 rotations. Polymerase chain reaction procedure advances to the final stage. This is the step of extension. During extension, the procedure of polymerase chain reaction is allowed to go on for a maximum of seven minutes (Zheng et. al, 45-12).

Once the process of template DNA amplification is over, the procedure for IRP1 identification and characterization goes ahead to the gel phase. This is the stage where the sample is run using 2.5 micro litre of the reaction on a gel (Michael, 88-74). This process is done to the final step which involves band visualization. During visualization there should be there should be a band corresponding to your polymerase chain reaction product. When the band is a strong primer dimer band which will demonstrate the primer-primer annealing is favored. This should not be the case for a good procedure (Zheng et. al, 45-12). In a standard procedure only the primer- template

annealing is encouraged (David, 34-47). This is because it will stop the formation of primer dimers. Primer- dimers are considered as noise in a PCR product. Noise is any unwanted component that will interfere with the reading of polymerase chain reaction product. Instances when there is a lot of noise in the product in the form of primer- dimers will interfere with the reading and the analysis of the Polymerase chain reaction product (New England Biolabs, 21-29). In this case there will appear to be a lot of product that is not useful to the analysis. This will cause the analysis to be inaccurate and incomplete (David, 34-47). This causes the results to be wrong because primer dimers do not form complete sets of primers that can be read.

The polymerase chain reaction product that is easy to read is that in which there is binding between the primer and the template forming a primer template combination. This primer –e template combination is what can be easily read for the analytical processes that follow after polymerase chain reaction (Baumann, 43-45). The analytical methods after polymerase chain reaction will include poly acryl amide gel electrophoresis which is mostly shortened as PAGE and also Spectrophotometry (David, 34-47). Spectrophotometry involves the analysis of the molecular weight and electrical charge of the proteins being examined (Dycke, 22-34). Spectrophotometry and gel electrophoresis can also be used to accurately identify and characterize the polymerase chain reaction products during analysis (Rouault, 67-43).

Visualization of the polymerase chain reaction products is done through microscopy. This is mostly by the use of the electron microscope and the dark field visualization. The visualization of the polymerase chain reaction product involved

viewing under the electron microscope (David, 34-47). During this process the samples are photographed and appear as band on the visualization element. These photograph analysis products are stored for records. It is these records that are compared to the sequences that are downloaded or searched from the protein data banks. It is after comparison of the invitro products that are sequences with the virtual products to come up with identity and characterization (Baumann, 43). After the visualization, photography and comparisons of the polymerase chain reaction products, there is digestion after which the last reaction is transformed into competent cells. This is followed by making the sequence into mini preps. The mini preps are used to check for any changes that have lead to mutations (Zheng et. al, 45-12).

Mutations occur when there are changes in the gene sequences. These changes occur by duplication, deletion and alteration process. These will either cause poly genes or reduced genes in the DNA strands (Michael, 88-74). This interference when inherited forms the parents, which can be the mother, the father or both parents to the offspring (Dycke, 22-34). When the offspring inherits these mutations they will have hereditary genetic conditions that they can pass to their offspring's depending on the inheritance and genetic factors. The procedures used can be used to also check for errors in the PCR procedure that could have caused alteration in results during the polymerase chain reaction process (Michael, 88-74).

The final process involves suspending the cells 0.2M sucrose, then suspending it in 100ml hepes at a pH of 7.4 then treat with 0.007 % digitonin. This process is carried out after which the sample is put in a centrifuge (Dycke, 22-34). The process that takes

place in the centrifuge occurs at 4° C. the centrifuge is set to rotate making 1800 spins per minute for every gram in the measurement of the sample. This entire process is allowed to take place for five minutes (Michael, 88-74).

The supernatants are afterwards collected and put in tubes. The supernatants are then allowed to spin in the centrifuge. This time the centrifuge is set to make 150000 rotations per minute and the procedure is allowed to go on until a total of 20 minutes. The supernatants collected are then stored at 80° C. these now constitutes the cytosolic extracts (Zheng et. al, 45-12).

The cytosolic extracts will then be taken through western blotting for further analysis. 10mgs of the extracts are for the protein lysates. These protein lysates are afterwards allowed to resolve on 10% SDS polyacrylamide gel electrophoresis. Then it is transferred to nitrocellulose membrane. This is allowed to set for an entire night. After setting for the whole night the sample is incubated (New England Biolabs, 21-29). During incubation an affinity rabbit serum that corresponds to the human compound is used. This rabbit serum is placed against the human IRP and ammonium terminal peptide (David, 34-47). Sample is also taken through northern blotting for ribo nucleic acid analysis. The final step involves Spectrophotometry. This occurs using bio Rad protein assay to analyze the sample by determining its charge and molecular weight (Pantopoulos, *Iron Regulating Protein*).

During the analysis of the results the concentration of primers and that of the templates is checked. This takes place after the entire process of polymerase chain reaction is complete (Pantopoulos, *Iron Regulating Protein*). The completion of

polymerase chain reaction will allow for the checking of the products of the polymerase chain reaction (Dycke, 22-34). These products are in form of primers. The expected results will come from the joining of template and primer strands. If there is any formation of primer to primer unions, the results are considered wrong (Rouault, 67-43). This is because, primer to primer annealing leads to formation of primer dimers. Primer dimers are considered noise when found in the final polymerase chain reaction product. Noise is any particle found in the polymerase chain reaction product. The noise particles arise when there is primer primer annealing (Dycke, 22-34).

The concentration of the final product of polymerase chain reaction in the characterizing of IRPs can be manipulated. This is done by reducing primer concentration while increasing the template concentration (Dycke, 22-34). The annealing temperature can also be changed to influence the polymerase chain reaction products.

Site directed mutagenesis involved linear amplification. This is because every time a new cycle is to take place in the polymerase chain reaction, the original template is the one that is used. This is unlike the standard polymerase chain reaction where by the primers formed are the ones used as parent template when the procedure is run a second time (Zheng et. al, 45-12). This is repeated for all the subsequent procedures (David, 34-47). In site directed mutagenesis however, the original parent template is the only one that is used for running the polymerase chain reaction to come up with polymerase chain reaction products (Dycke, 22-34).

In site directed mutagenesis, the product is digested using a product called, DpnI. This will involve placing the sample in 0.25 micro litre of DpnI. The sample is then put in an incubator which is set to a temperature of 37° Celsius. The incubation process is carried out for a total of one hour (New England Biolabs, 21-29). A process called high fidelity polymerase will minimize the occurrence of unwanted changes in the genetic makeup of the iron regulatory protein that is being observed (Rouault, 67-43).

Conclusion

Iron regulatory proteins (IRPs) control the translation of proteins involved in iron uptake which occurs after an intake of iron mineral in the meal. The iron regulatory proteins then go ahead to take part in the regulation of iron storage in the human body. Storage of iron is in the form of an iron compound called ferritin. After storage the iron has to be moved through the body to the source where it is utilized. This occurs by use of binding factors. Iron is transferred from its source to the place of utility by use of binding factors. These are found in sites of reception where the iron receptors bind the iron compounds and transport them to the final destination where they are utilized. Iron rich compounds in the body are mainly utilized in the formation of red blood cells. This is specifically the formation of the heme compound found in the hemoglobin part of the red blood cells. This process occurs in the final stage of iron movement in the blood. The use of iron in the body is through the compound formation of erythroid alpha- amino/ aevulinic acid synthesis.

The procedure for the characterization of the iron regulatory proteins starts with the formation of primers called primer design. These primers called oligonucleotides will

be used in the polymerase chain reaction procedure that entails amplification of the parents strand. Hence the step that follows is the polymerase chain reaction procedure that amplifies the small parents' template into a substrate that can easily be observed and analyzed. The polymerase chain reaction step was followed by the gel electrophoresis step. In the gel electrophoresis step, the main gel that is used is the agarose gel for analysis of the polymerase chain reaction products.

Flow charts

Figures 3, a, b and c: Modulation of IRP1 (IRE-Binding Process):

Figure 3.a.

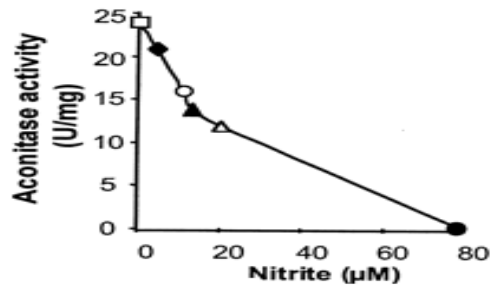


Figure 3.b.

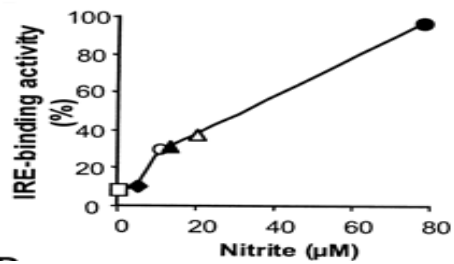
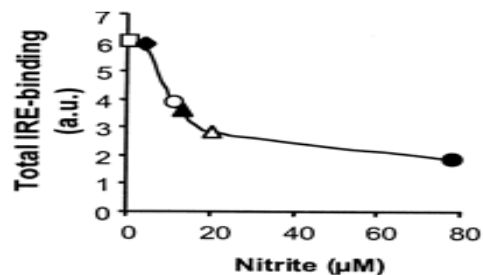


Figure 3.c.



Reference: Oliveira, Leonor & Jean Claude Drapier. "Down Regulation of Iron Regulatory Protein 1". University of California, Los Angeles, CA. 2000.

Figure 4.a and b: Down Regulation of IRP1 (In RAW 264.70 cells exposed to IFN- γ)

Figure 4a

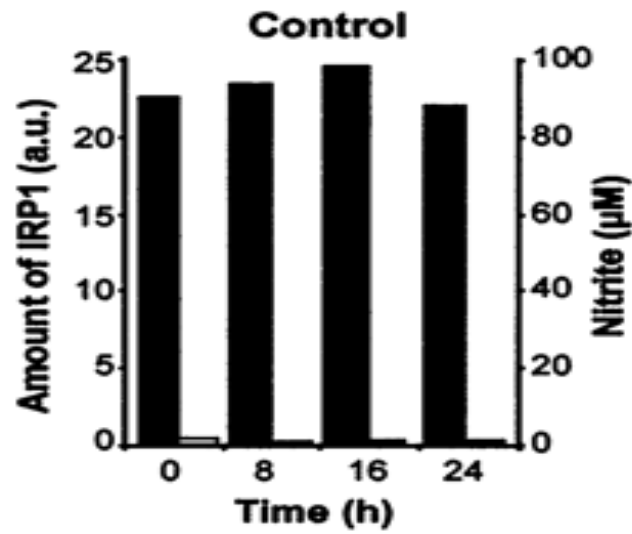
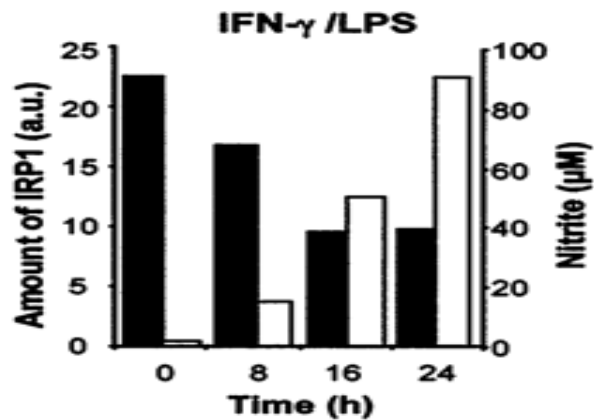


Figure 4b



Reference: Oliveira, Leonor & Jean Claude Drapier. "Down Regulation of Iron Regulatory Protein 1". University of California, Los Angeles, CA. 2000.

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