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Induced Pluripotent Stem Cells

Gene-Editing on Transthyretin Amyloid

Highlights

Cerevisiae in Galactose Environment

Scientific and Research Organizations

Discovering Thoughts, Inventing Future

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Induced Pluripotent Stem Cells and CRISPR-Cas9 Gene-Editing on Transthyretin Amyloid Cardiomyopathy

By Caden L. Reedy

Abstract- Transthyretin amyloid cardiomyopathy is a fatal disease of the myocardium causing a protein buildup of Transthyretin. Over 120,000 people in the United States suffer from transthyretin amyloid cardiomyopathy, and half of those diagnosed will die within four years of the onset of symptoms. However, applying CRISPR-Cas9 gene editing will reduce amounts of transthyretin produced by the liver by up to 96% and minimize transthyretin expression by 91%. Induced Pluripotent Stem Cell therapy shows signs of at least 20 years or greater in life expectancy. It puts 39% of recipients into complete remission. Using CRISPR-Cas9 technology, an IV is placed and lipid nanoparticles deliver mRNA with Cas9 production and a single guide RNA for targeting the transthyretin production in hepatocytes. Gathering stem cells has never been easier, using adult somatic cells and returning them to an embryotic state is more efficient and ethical than ever.

Keywords: *Pluripotent, Stem Cells, Cardiomyopathy, CRISPR, Gene Editing, Amyloidosis, Treatment, Heart Disease, Cas-9.*

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Induced Pluripotent Stem Cells and CRISPR-Cas9 Gene-Editing on Transthyretin Amyloid Cardiomyopathy

Caden L. Reedy

Abstract- Transthyretin amyloid cardiomyopathy is a fatal disease of the myocardium causing a protein buildup of Transthyretin. Over 120,000 people in the United States suffer from transthyretin amyloid cardiomyopathy, and half of those diagnosed will die within four years of the onset of symptoms. However, applying CRISPR-Cas9 gene editing will reduce amounts of transthyretin produced by the liver by up to 96% and minimize transthyretin expression by 91%. Induced Pluripotent Stem Cell therapy shows signs of at least 20 years or greater in life expectancy. It puts 39% of recipients into complete remission. Using CRISPR-Cas9 technology, an IV is placed and lipid nanoparticles deliver mRNA with Cas9 production and a single guide RNA for targeting the transthyretin production in hepatocytes. Gathering stem cells has never been easier, using adult somatic cells and returning them to an embryotic state is more efficient and ethical than ever. Can these two treatments combined cure and improve life expectancy as well as quality of life in patients with transthyretin amyloid cardiomyopathy? These two combined will decrease symptoms that patients have as well as increase the quality and longevity of life. People going into remission on a previously thought impossible disease is remarkable, and using the advancements of CRSIPR-Cas9 and Induced Pluripotent Stem Cells together to solve transthyretin amyloid cardiomyopathy is the future of the treatment plans for the disease.

Keywords: Pluripotent, Stem Cells, Cardiomyopathy, CRISPR, Gene Editing, Amyloidosis, Treatment, Heart Disease, Cas-9.

I. Introduction

Over 120,000 Americans have Transthyretin Amyloid Cardiomyopathy (ATTR-CM), one in 2700 Americans, 5000-7000 new cases a year, men seven more times likely than women. (Institute for Clinical and Economic Review, 2024). A haunting disease with an extremely poor prognosis, but usually goes undiagnosed and leads to heart failure. (*Transthyretin Cardiac Amyloidosis | ATTR-CM for HCPs | Therapy Area*, n.d.) When a patient in the past was given a diagnosis, it was usually a liver or heart transplant, and sometimes an LVAD (Left Ventricle Assisting Device) which are all surgical and extremely high risk. In the four years after the onset of symptoms, half of the patients will die. To explore treatments and possibly cures, I have looked at one of the leading treatments that show extreme recovery and remission and a new promising gene editing drug. I saw the existence of both treatments to be together and work hand in hand. The disorder thickens the wall of the left ventricle, making it harder for adequate pumping function, reducing ejection fraction rate, and being put at risk for heart failure. The main risk factor for developing ATTR-CM is HCM (Hypertrophic Cardiomyopathy), this genetic condition causes the wall of the heart to become thickened as well and can cause heart failure. Induced Pluripotent Stem Cells (IPSCs) as well as new CRISPR gene editing can completely eradicate the symptoms and possibly even the disease entirely; both treatments are showing promising signs separately and together they could work together in unison without any negative interactions or interferences. 39% of patients who received stem cells alone were in total remission (*BU Study: Stem Cell Transplantation for AL Amyloidosis Leads to Long-term Survival and Possible Cure in Selected Patients | Clinical & Translational Science Institute*, 2022). I hypothesized that two treatments with good outlooks can work together and treat transthyretin amyloid cardiomyopathy.

Author: Oak Grove High School. e-mail: cadenlreedy@gmail.com

II. Materials and Methods

a) Study Design

Patient statistics were monitored with IPSCs and without them as well as monitoring patient statistics and outcomes with CRISPR-Cas9 and without from 1994-2024. The two treatments were never given to the same patient in the same care plan but were compared from treatment to treatment. Statistics were monitored and documented from outside sources in citations. Patients received one dose of CRISPR gene editing through IV between November 2020 and April 2021. Patients were either given 0.1mg per kg or 0.3mg per kg of RNA dose. Patients were aged 18-80, with a range of weight between 50-90kg (Gillmore et al., 2021). IPSCs were given to specific patients and statistics were done on life expectancy, state of disease, and prognosis. This study was conducted in Auckland, New Zealand. (*BU Study: Stem Cell Transplantation for AL Amyloidosis Leads to Long-term Survival and Possible Cure in Selected Patients | Clinical & Translational Science Institute*, 2022).

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b) Ethics Review

Ethics are usually a sensitive topic in stem cell research because they are derived from a human embryo, Induced Pluripotent Stem Cells are adult somatic cells taken from skin or blood (not embryotic) but were taken to the lab and going through cellular reprogramming by erasing and reshaping and remodeling the cells "differentiation memory". This can happen by expressing four transcription factors: Oct4, Sox2, Klf4, and c-Myc. This brings the cells into an undifferentiated embryotic form without using or being an embryo. The main disadvantage of this on the ethical side would be that this could turn into an actual embryo if exposed to the correct conditions.

III. Results

An induced knockout was the result of the CRISPR-Cas9 gene editing. Patients who received 0.1mg per 1kg had a reduced transthyretin concentration of 52%, and patients who received 0.3mg per 1kg had a reduced transthyretin concentration of 87%. Over 95% of transthyretin production was halted, 91% of transthyretin expression was reduced and showed few adverse effects. 12 months after the mRNA was delivered to the hepatocytes the production and expression of transthyretin was still maximally suppressed (Gillmore et al., 2021). Boston University researchers say hematologic complete remission was achieved in 39% of patients with a median survival of 15 years and 30% of those patients survived longer than 20 years (after onset of symptoms). A permanent reduction of transthyretin production and concentration was claimed. (*BU Study: Stem Cell Transplantation for AL Amyloidosis Leads to Long-term Survival and Possible Cure in Selected Patients | Clinical & Translational Science Institute*, 2022).

IV. Discussion

The results show a promising future and do support my hypothesis that the two treatments together would be beneficial and treat patients with ATTR-CM better than they have ever been. Patients going into remission, living longer, and having a better quality of life is something we all should strive for. Results show that separately the treatments are working extremely well. The growing understanding of gene editing, stem cells, and diseases, will come up with new treatment plans and objectives, where we once wanting to give medications for symptoms to go away, to now giving stem cells and CRISPR gene editing and truly having hope for remission in people suffering from this. Using the two treatments in unison as one treatment plan will save lives and be revolutionary to the treatment of cardiomyopathy. 39% of patients in remission with one of the treatments alone is incredible, this fully supports my hypothesis, there is no room for misunderstanding when numbers like these are being produced. Stopping the production of transthyretin proteins with CRISPR-Cas9 will cause the protein to be restricted and the stem cells will repair and enhance cardiac tissue in need.

Past treatments have never come close to the current treatments. With current technology, we can access and do things we have never imagined happening. Once we used stem cells from embryos and now, we can derive stem cells from skin and blood cells. Our technology and field have grown tremendously. Treatments for transthyretin cardiomyopathy have never been better. This is the first time we have seen patients completely cured of the disease. Not being on lasting drugs, having impairments, symptoms, and living restricted lives. When these two treatments are used together, they will without a doubt change the course of treatment for ATTR-CM.

V. Conclusion

The results of the two studies support my hypothesis undoubtedly. I can see a future where we have cures for diseases like this one, where a diagnosis is not a sentence, where we have a chance. These treatments open a window for that opportunity.

VI. Declaration

I, Caden L. Reedy declare that research 'Induced Pluripotent Stem Cells and CRISPR-Cas9 Gene-Editing on Transthyretin Amyloid Cardiomyopathy' has not been published nor is being reviewed elsewhere.

I, Caden L. Reedy declare all the following:

Ethics Approval: Yes.

Consent for publication: Yes.

Availability of Data and Materials: Yes.

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Authors Contributions: Author took lead in writing all manuscript, gathered and organized all data, and came up with the research idea.

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NET-seq and MNase-seq Analysis of Gene Response of S. Cerevisiae in Galactose Environment

By Venkata Adiya Satvik Bhamidi

Introduction- Earth has diverse and extreme environments so, for an organism to survive, it is vital that it is able to respond to changes in temperature, availability of food and even other organisms. For organisms to function properly, and adapt to these conditions, gene expression at the right time is essential. Gene expression is the process by which information in DNA is transferred to RNA molecules that create proteins for various functions. The control over the creation of proteins for a situation is essentially what gene expression and gene regulation is1. For instance Himalayan rabbits that were bred at temperatures less than 20 C had black sections of fur, while those bred at 35 C or more had completely white fur, suggesting differentiated gene expression for a changed condition- temperature2. The first step of gene expression is transcription, therefore measuring the levels of transcription of genes in different conditions can be an indicator of the effects of gene regulation. In order for transcription to occur, mRNA segments, named “transcripts”, are copied from DNA, so finding which mRNA transcripts are copied can indicate gene expression for various times and conditions. SAGE and Northern Blot techniques measure mRNA segments to analyze gene expression..

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Venkata Adiya Satvik Bhamidi

I. Introduction

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The organism analyzed in this paper is yeast, more specifically the Saccharomyces cerevisiae species, or baker's yeast. This organism is well studied in the genetics field, due to its similarity to human cells, as it is eukaryotic and has homologous proteins to that of mammals. It is also the first eukaryotic organism to be fully DNA sequenced[6], having 16 chromosomes and 5331 actual protein coding genes[7]. It is also very cost effective and easy to acquire, since it can be found in almost any store both scientific and general. This paper will focus on the changes in the yeast's gene response and such when presented with a changing environment- in this case to have a different source of food. This test could be significant because yeast can utilize glucose better than galactose, which they need to add enzymes and such to modify as a viable food source. One such change can be the gene expression of the GAL group that regulates the metabolism of galactose that is not activated in a glucose environment. When galactose is the sole carbon source, the galactose-metabolizing enzymes are expressed at 1000 times their level in glucose[8].

This paper will measure the overall effects on gene expression in S. Cerevisiae with the addition of a galactose environment.

Author: Department of Biology, Cypress Bay High School.
e-mail: satvikbhamidi@gmail.com
ORCID: 0000-0002-4544-6220

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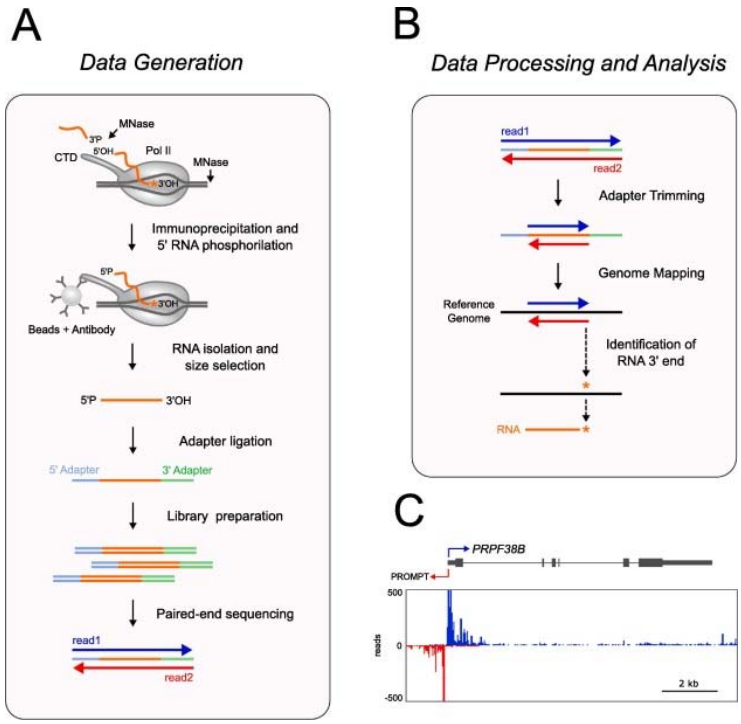

Figure 1: The schematic diagram of the NET-seq method. Reference: See Figures section of References

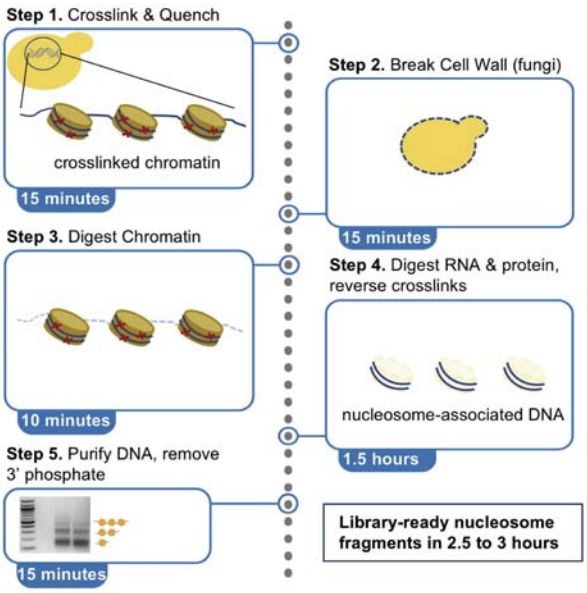

Figure 2: The schematic diagram of MNase seq method Reference: See Figures section of References

II. Method

a) Preparation of Saccharomyces Cerevisiae

The yeast were originally placed in a glucose environment to be intended as the control group. Yeast was then cultured in the glucose environment for two hours under a constant temperature of 37 deg. C. Then samples of S. Cerevisiae were transferred into the galactose environment, for5,15, and 60 minutes to see the changes in gene expression when a change in environment and food source was presented, with each of the time periods in which the samples were collected tobe the experimental groups.

b) Acquiring Data

Saccharomyces cerevisiae was prepared for and NET sequenced, for when the samples were in glucose, and sequenced periodically when the yeast was placed in galactose for 5 minutes, 15 minutes and 60 minutes. MNase sequencing was also prepared for and performed on the yeast when they were originally in a glucose environment and when they were in the galactose environment for 60 minutes.

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c) Analyzing the Data

The sequencing data was analyzed in MATLAB. First, an average gene profile was created to show the typical behavior of all the genes, named metagene. This allows for greater insight aboutthe overall trends across all the base pair positions, In order to find the genes that have had a significant change from switching to galactose environment, a t-Test with significance of 0.05 was performed on each of the meta gene datasets. The gene profiles of genes that responded to the change of environment (category: responsive genes) were compared to the genes that did not (category: non-responsive genes). The chromatin and promoter information of these two categories of genes was further investigated using the MNase sequencing dataset.

d) Antisense Transcription

Antisense transcription was also considered and the transcription levels for responsive and nonresponsive genes were graphically represented, in both sense and antisense genes, for both NET-seq. The antisense transcription levels were compared with the sense transcription levels, in the responsive and non responsive genes. The promoter regions of the sense and antisense transcriptions were also analyzed for differences. To find the promoter region, only the first 100 genes were graphed as the data started from a position of about -1000 bp to 500 bp of the transcription starting site (TSS). Therefore, the promoter would be somewhere in the region from
-1000 to 1bp.

III. Results and Discussion

A glucose environment is S. Cerevisiae's natural environment, as it is their primary and most efficient source of energy. The presence of glucose suppresses metabolic activity to perform gluconeogenesis, or to synthesize glucose from other carbon compounds, with the use of signaling. These processes control the transcriptional and translational activities, especially with the Snf3/Rgt2 glucose-sensing pathway and the Snf1 signal transduction[1]. Galactose was chosen for the experimental group because the yeast cells cannot directly perform glycolysis to gain energy like in glucose, but S. Cereviase and other species of yeast utilize GAL genes, which are activated only in the presence of galactose to regulate transport and metabolism of the sugar, and have to modify galactose using enzymes to use as food[2], thus opening the potential to see varying degrees of gene expression changes with the difference in environment.

Out of S. Cerevisiae's 5331 genes, the t-test of the meta-genes resulting from the NET-seq data ofthe S. Cerevisiae (Appendix section 1,2) with a significance level of 0.05 showed 4849 genes responded to the change in environment from glucose to galactose in 5 minutes. 4918 genes responded to change in environment in 15 minutes and in 60 minutes 4756 genes responded to changes. There were 4141 genes that responded in the above 5-minute, 15-minute and 60-minute groups, therefore about 78.7% of the total genes were considered to be active to the environment change. Figure 3 shows the mean NET-seq levels of all the genes, regardless of the activity levels. The similarity of the graphs of the galactose mean NET-Seq levels to that of the glucose graph across all the genes decreases as more time is spent in the galactose environment- this change is most apparent at the beginning of 15 minutes of being in the galactose environment, indicating approximately the time when the genes started to respond to galactose. Figure 3 further demonstrates that the genes located between -200bp to -100bp relative to TSS and 700bp to 750 bp relative to TSS have increased transcription the most.

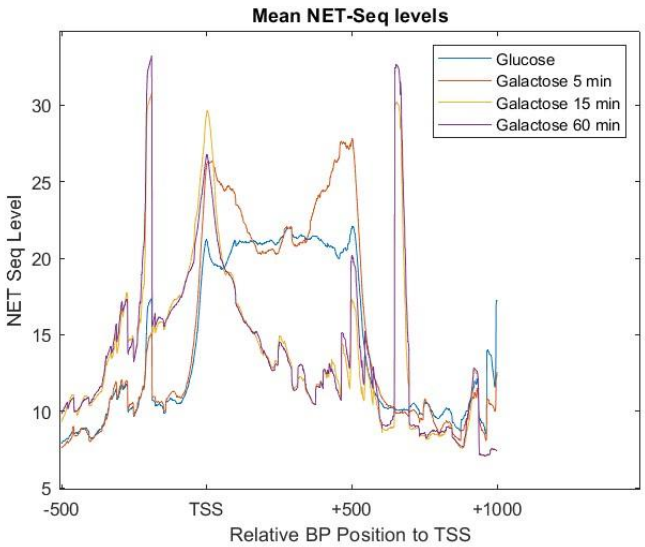

Figure 3: The average NET seq levels for S.Cerevisiae inglucose and galactose environment for all time intervals

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Focusing the transcription differences between the glucose and galactose 60-minute group, Figure 4 suggests that the responsive gene showed a higher NET-seq level consistently than when it was in a glucose environment, thus it was activated and more transcription had occurred when it was in galactose. This gene in particular helps to control lipid and amino acid metabolism in the yeast [3], thus it being activated will help the yeast get such nutrients from the galactose environment.

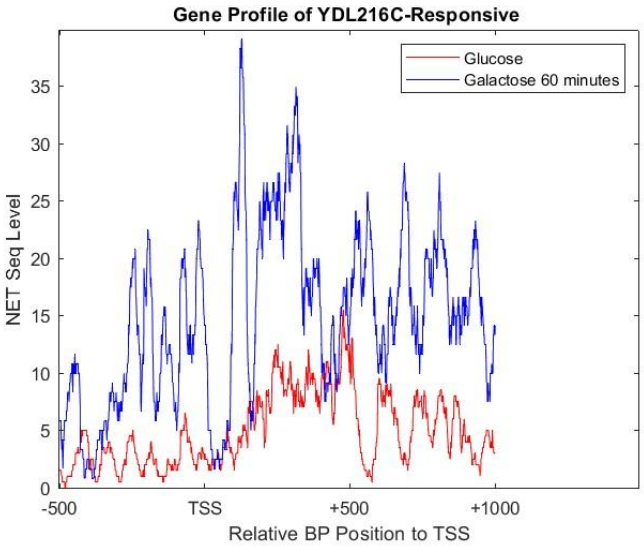

Figure 4: Gene profile based on NET-seq levels of YDL216C,which responded to galactose in 60 minutes of exposure

On the other hand, as shown in Figure 5, the gene YOR255W was a nonresponsive gene that had a very low net seq level when it was in a glucose environment. It does not show consistently high net seq levels when exposed to the galactose environment, even having no NET-seq levels periodically, such as between 0-200 and 600-800. This gene is responsible for helping create protein for the outer spore walls for the yeast during mitosis [4]. Therefore, it logically, does not have that much of an active role when the yeast is transferred to a galactose environment, as it only activated in some stage of mitosis, but it's periodic rise and fall in net-seq levels does indicate when the yeast is undergoing mitosis, especially seen between 800-1000bp.

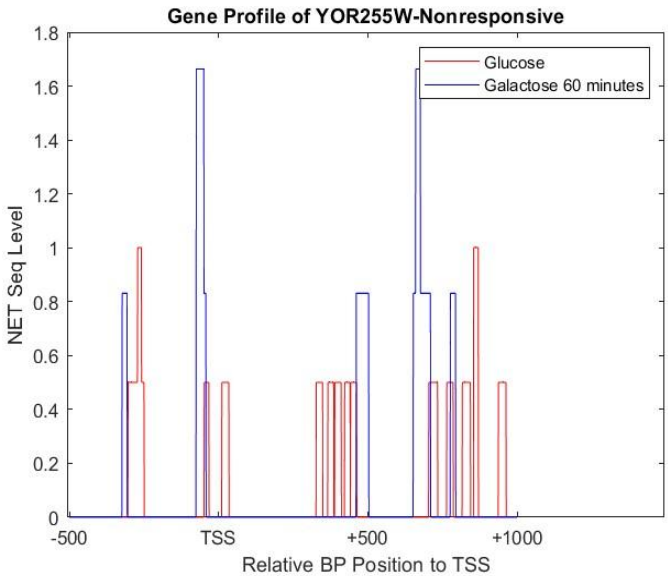

Figure 5: Gene profile based on NET-seq levels of YOR255W,which responded to galactose in 60 minutes of exposure

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Antisense transcription is generally lower than the sense transcription due to promoters of the antisense strand being less noticeable and less transcript able than the sense promoters[5]. It still is important to gene expression as they can quickly respond and integrate with various stimuli, and variate quickly sending the genes into on or off positions[6]. They thus can allow for greater survivability of an organism in different environments overtime due to evolution, and must be studied further due to their relative uncertainty over why they function the way they do. The levels of transcription for sense and antisense genes in the 60 minute interval for the galactose environment were compared in Figure 6. According to the acquired NET-seq data, the levels of transcription of responsive genes are inverse of each other, where a high level of transcription in the forward strand corresponds to a lower level of transcription in the reverse strand. This is apparent especially in the 200-800 region.

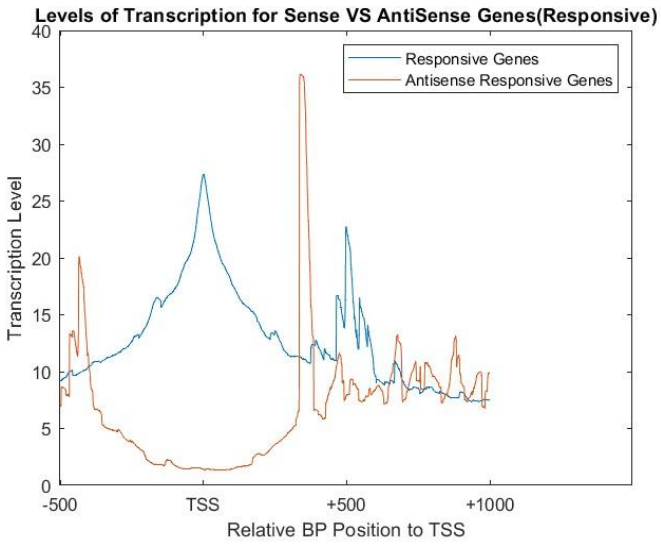

Figure 6: Levels of transcription in genes that responded to galactose in 60 minuteinterval in both sense and antisense direction using NET-seq data

Figure 7 shows the levels of transcription for sense and antisense genes in the 60 minute interval for the galactose environment. These genes generally have a lower transcription level than the responsive genes, and generally even less antisense transcription levels. This was hypothesized since the nonresponse genes remain dormant as they are not affected by the change in environment.

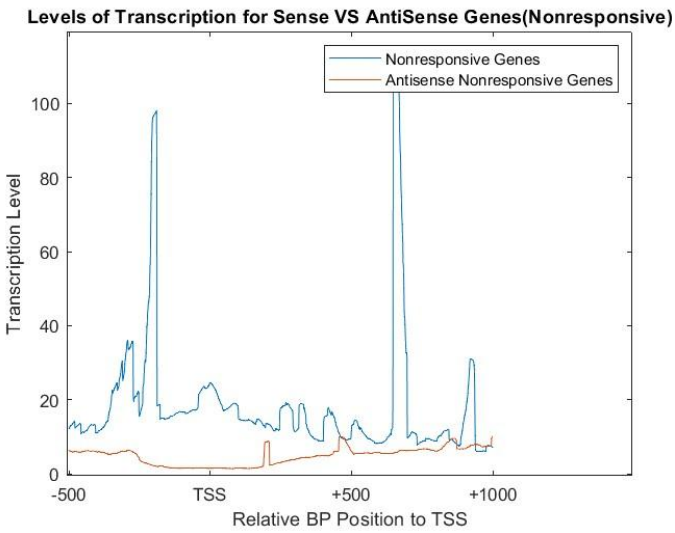

Figure 7: Levels of transcription in genes that did not respond to galactose in a 60 minute intervalin both sense and antisense direction using NET-seq data

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Figure 8 shows the MNase seq levels of the responsive and nonresponsive genes. The promoter is approximately between -355 to -400 bp relative to the TSS, as it is indicated by lower than usual MNase seq levels. The overall MNase seq levels are very consistent with the responsive and non responsive genes. The location of the histone proteins are also evident, beginning at about relative to 500bp to the TSS, where there is a regular sinusoidal pattern where a higher MNase-seq signal indicates less transcription, blocked by the DNA being wrapped around the histone, and lower levels are where DNA strands likely will be transcribed, as the region is between the histone proteins.

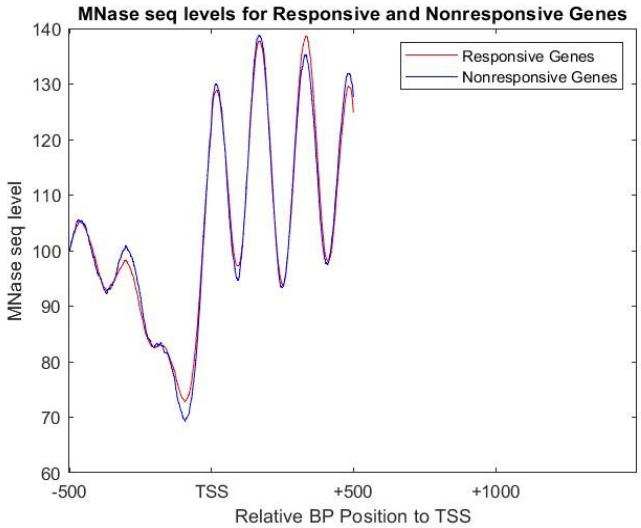

Figure 8: MNase seq levels for both responsive and non responsive genes when exposed to 60minutes of a galactose environment, in order to identify the possible promoter region

Additionally, histone modifications can also play a role in gene regulation in yeast- specifically for those genes that did respond to galactose. These processes can affect various chromatin related activities like DNA repair[7] and can do this by repressing or activating transcriptional activity. The exact effects depend on the type of histone modification under question, the organism and the genes that have been affected. However, various studies describe that histone methylation, a type of histone modification, does correspond with increased gene expression along with histone acetylation, along with DNA acetylation. In S.cerevisiae specifically, the histone acetylation depends on the transcription levels of the yeast by targeting the nucleosomes where RNA polymerase II accumulates[8], but in some instances the process itself is needed to facilitate transcription in yeast- by regulating bonds between histones and DNA.

IV. Conclusion

The transition from a glucose to a galactose environment has various effects on S. Cerevisiae, especially on a genetic level, with various aspects of gene expression such as activation of genes, transcription levels and antisense activity all have changed. Overall, the results point out that there is more of such gene expression when the yeast has switched to a galactose environment, and these levels have increased as more time was spent in the galactose environment, indicated by the number of genes (nearly 80%) that have responded to the galactose environment, the average net-seq levels and the gene profiles. Antisense transcription has been higher for responsive genes than in non responsive genes. These results are logical as the galactose environment forces the yeast to perform gluconeogenes is and other processes that require many of the genes to be activated to carry out regular functions that do not need such higher levels of activity in its natural glucose environment, which does not need modification.

Further research can be done on this organism's gene expression, for instance utilizing various other techniques such as CHip-seq data to gain more information about histone modifications, and RNA-seq for final transcript levels. There can also be more environments in which the S. Cerevisiae can be placed in, such as sucrose or lactose, or a combination to find how gene expression changes. The amount of time spent in these environments could also be changed. Such analysis can provide various insights into the changes larger, eukaryotic organisms, especially people can respond to on a genetic level. This can further the understanding of how genes regulate human function, and how problems with the regulation in the presence of a changing environment can create better health treatments such as more accurate and individualized diet plans, better screening techniques that can detect

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genetic irregularities with various stimuli and predict risks for patients.

Limitations of the Study

Our research was limited due to time and resources for data analysis, which resulted in no CHIP-seq test analysis and also testing of histone modification behavior with transcription. It is recommended to run tests using these parameters to increase data collection range.

Ethical Approval

No ethical approval was needed for this study.

Informed Consent

Informed consent form was submitted to the journal.

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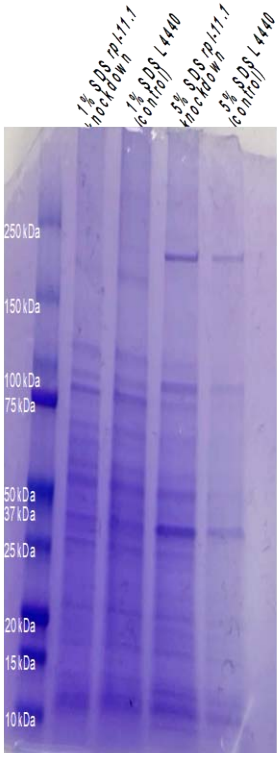

Figure 1. 1% and 5% SDS-PAGE of proteins collected from *rpl-11.1* knockdown and L4440 (control) *C. elegans* PP563. The gel was imaged by smartphone 48MP camera at ISO 200. Contrast has been adjusted for presentation clarity.

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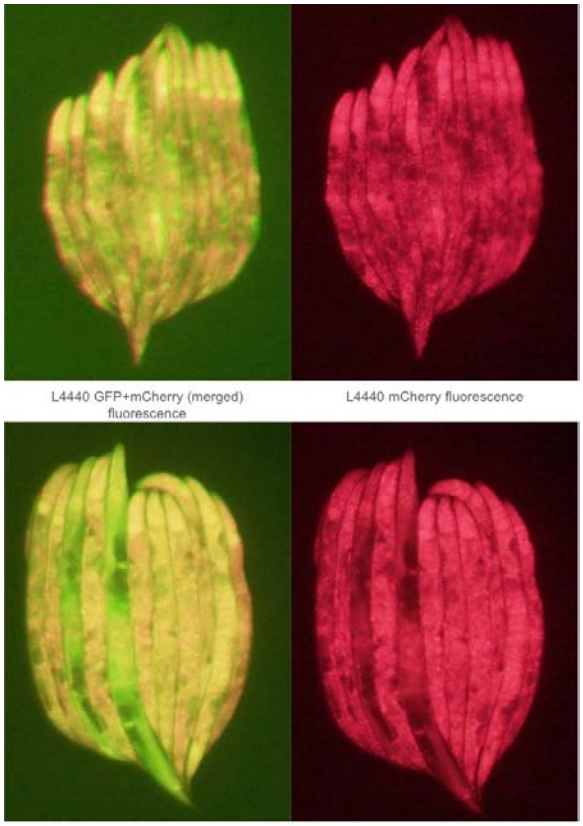

Figure 2: *rpl-11. 1* knockdown in MAH215 via RNAi in comparison with L4440 control. Micrographs taken at 31.6ms exposure time and 2.0x gain.Merged fluorescence images are acquired by combining coloured images (GFP signal and mCherry signal) *in silico*

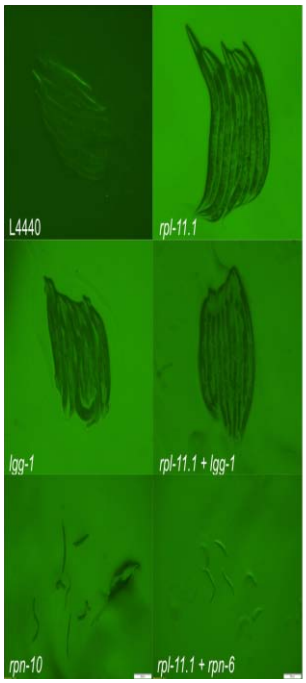

Figure 3. Micrographs of double-gene knockdown *C. elegans* PP563. Micrographs were taken at 31.6 ms exposure and 2x gain.

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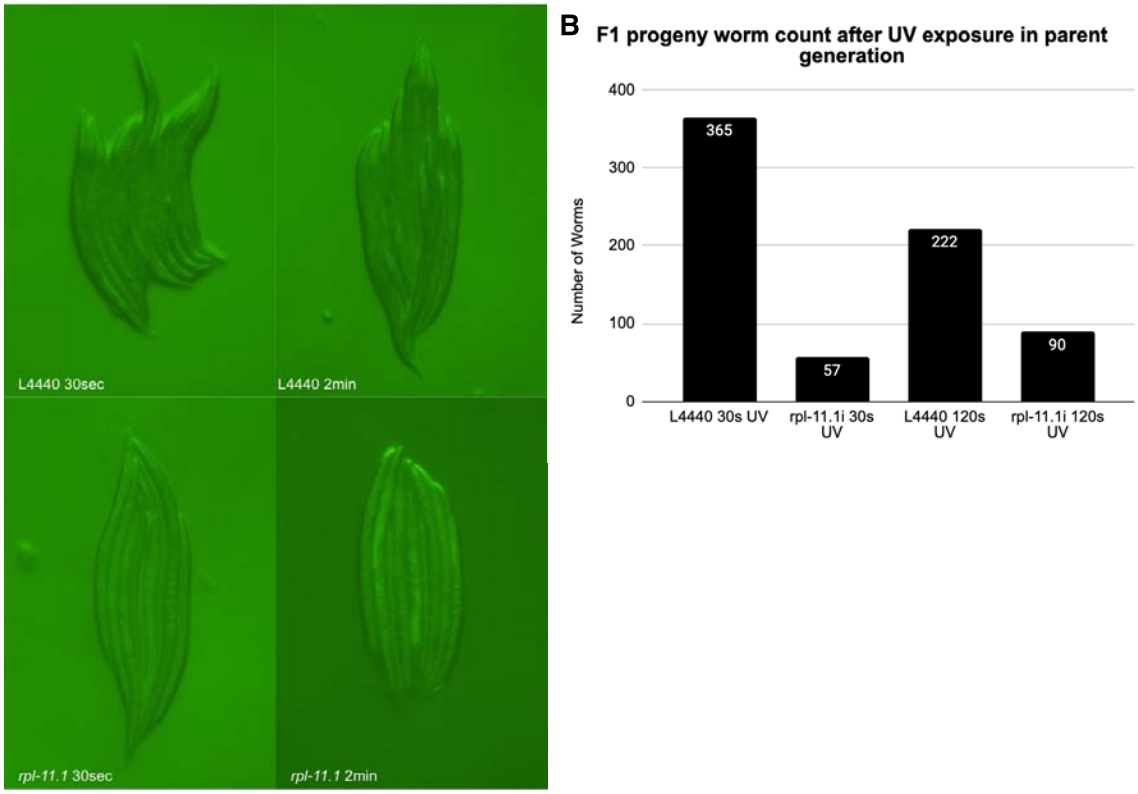

Figure 4: The F1 progeny of UV-treated nematodes. **A.** Micrographs of the F1 progeny of UV-treated nematodes. 10 worms from each group were randomly selected, aligned and immobilized. Micrographs were taken at 31.6 ms exposure and 2x gain. **B.** Worm count of F1 progeny after UV treatment in parent generation. L4440 and *rpl-11.1* knockdown *C. elegans* PP563 adults are exposed to 30s or 120s UV radiation in gel imaging chamber. 5 worms from each group are transferred to new plates. Worm count was conducted after 3-day incubation at 25 °C

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On the Guard of World Peace. Scientific and Research Organizations in NATO Structures 1952-2022. Problem Outline

By Janusz M. Ślusarczyk

Abstract- Scientific research organizations have a long-standing tradition in NATO's history, testifying to the great role that Alliance decision-makers attach to scientific research. Thanks to comprehensive scientific development, the existence of specialized scientific institutions and cooperation with research units of member states, it was and is possible to develop and grow NATO's power. Thanks to scientists, the Alliance has for many years been a world leader in the development and application of modern military technology. The assembly and cooperation of leading representatives of the scientific and technological world of the member countries has made it possible to develop and implement many innovations in the field of defence. With the passage of years, it has become necessary to develop specialized scientific and research institutions, conducting new research and addressing further challenges facing the defence policy of the Alliance.

Keywords: *NATO, science, AGARD, DRG, RTO, STO, SPS, SACLANTCEN, NURC, CMRE.*

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NATO's first scientific research organization was AGARD, dedicated to aerospace research and the development of scientific cooperation. The goal of the DRG, established in 1967, was to promote technical cooperation among allied countries in research and new technologies, leading to the development of defence equipment. From the merger of AGARD and DRG, the RTO was formed, operating in the field of defence research and technology. Since 2012, STO has been operating to meet the scientific and technological needs of the Alliance. In 1996, NM&S was founded, developing Alliance approaches to simulation to improve operations. NATO has also created specialized maritime scientific research organizations. SACLANTCEN, NURC and CMRE were active between 1959 and 2022, conducting research in anti-submarine warfare, underwater acoustic phenomena and their application in surveillance, detection, oceanography and port security. NATO's scientific research organizations make a significant contribution to strengthening the Alliance's security and defence forces.

Keywords: NATO, science, AGARD, DRG, RTO, STO, SPS, SACLANTCEN, NURC, CMRE.

I. Introduction

Scientific and research development is the main driving force behind societies and modern civilization. Since the beginning of the scientific and technological revolution (the third industrial revolution) initiated in the 1950s, there has been a rapid development of science and technology in highly civilized societies. This was an international trend, the result of the Cold War arms race and, at the same time, the activities of large corporations operating in oligopoly conditions and competing with each other in the field of new products. During the Cold War, seeing the need to develop the Alliance, its leaders realized the need to undertake scientific research aimed at strengthening the pact's forces. The establishment and development of NATO's scientific research institutions was a response to these demands.

Analysing the activities of these institutions, it should be stated impartially that it has brought a significant impact on the increase in combat readiness of the Alliance forces. Its distinctive feature is the great reduction in the duration of the process from the inception of an innovation to its implementation. Many cutting-edge technologies were developed and incorporated, including information processing, environmental protection, new equipment and hardware. It was important to create platforms for cooperation between scientists from Alliance member countries, so that the flow of ideas and experience became possible. Today, research focuses on defence issues on land, sea, air and space.

The presented text aims to present the history and present day of the activities of NATO's scientific and research institutions. For its purposes, a research hypothesis was formulated: the development of NATO's scientific and research institutions has made a significant contribution to the work of strengthening the defence of the Alliance. The questions posed were: how did the various organizations contribute to the realization of NATO's tasks, and how did the Alliance's policy on the development of these organizations change. The text consists of subsections showing the activities of individual scientific research organizations, presented in a chronological context.

II. Methodology

The basic research material (source materials) was information obtained from official NATO documents, published on websites. They concern, among other things, the activities of individual organizations and reports of the Alliance. These materials are fully reliable

Author: Department of Internal Security, High School of Administration in Bielsko-Biała, Poland. e-mail: janusz8639@gmail.com

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and show as fairly as possible the activities of the organizations discussed in the text. In addition, book items were used in several places. Critical analysis of these materials made it possible to compile the text and formulate answers to the questions posed.

III. Results

a) AGARD

NATO's first scientific research institution, the Advisory Group for Aerospace Research and Development (AGARD), was established in 1952. It had four sections (cooperation, aeronautics, flight test and instrumentation, and wind tunnel and model tests). The task was to promote and improve the exchange of information on aeronautical and space research work and development among NATO countries. AGARD also provided scientific and technical advice and assistance to the NATO Military Committee in the field of aerospace research and development, with particular emphasis on military applications. The number of scientists grew from 100, to 200 in 1960 and more than 500 in the 1990s. AGARD's highest authority was the National Council of Delegates, consisting of appointed representatives from each member country (Van J. A. 2001). The organization aimed to bring together leading scientists of NATO countries in the fields of science and technology related to aerospace in the following topics:

- Recommending effective ways for member countries to use their research and development capabilities for the mutual benefit of NATO,
- Providing scientific and technical advice and assistance to the military,
- To stimulate progress in aeronautical science related to defence strengthening,
- Improving cooperation among member countries in aerospace research and development,
- Exchanging scientific and technical information,
- Providing assistance to member countries to enhance their scientific and technical capabilities.

Participation in the sections generally lasted three years, although it could be extended. The specific areas of interest of each section changed relatively quickly with the passage of time and technological advances. Each section determined the research and publication program in its specialty, within the general AGARD guidelines set by the Board of Directors. The result of the work was, among other things, the publication of 70-90 scientific papers per year.

As document analysis testifies, during its existence AGARD organized the exchange of scientific information of military significance. Their goal was to strengthen NATO's defence forces and enhance the scientific capabilities of member states. The organization evolved over the years and consisted of a Council of Delegates, reporting to the NATO Military Committee, and thematic sections. The Council of Delegates provided guidance to the sections and approved their work program. In January 1998, AGARD and DRG merged.

b) DRG

NATO's second science and technology organization, the Defence Research Group (DRG), was established in 1967. It was realized that, despite the end of the Cold War, the development of research contributes to the defence of the Alliance's forces. The main emphasis was intended to be placed on the development of international cooperation. This idea was very correct, as it allowed for increased exchange of scholars and a wider and faster flow of information.

Unlike AGARD, which focused on research, the DRG's main objective was to foster technical cooperation between NATO countries on research and new technologies. The DRG consisted of some 500 outstanding specialists from each Alliance country. They specialized in defence research and development. They worked within the framework of eight sections and two special expert groups. Scientific and research issues included:

- Physics and electronics,
- Optics and infra,
- Operations research,
- Human and biomedical sciences,
- Electronic warfare,
- Air warfare,
- Information processing technology,
- Camouflage,
- Combat engineering technology (AGARD, RTO and STO History, 2020).

An analysis of the source materials reveals that during the 1990s, there was a growing recognition within NATO that there was unnecessary duplication of effort between the DRG and AGARD. There was also some concern about the number of people involved, which had grown to more than 1,000 scientists, engineers and administrators. As a result, NATO Secretary General Javier Solana disbanded the DRG and AGARD in April 1997 as part of a restructuring of defence research and technology. The NATO Research and Technology Organization (RTO) was established, taking over the responsibilities of its predecessors (Daniel, Caraher, 1-6).

c) RTO

The RTO began operations in January 1998, with the NATO Research and Technology Agency (RTA) as its executive body. The RTO was NATO's main defence science and technology organization. It promoted and conducted research and information exchange, developed and maintained the Alliance's long-term research and technology strategy. It was also

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involved in advising NATO members on research and technology issues. The organization promoted cooperation between Alliance bodies and NATO member and partner countries to maximize the effective use of modelling and simulation (Proceedings of the 1st NATO Research and Technology Organization (RTO), Human Factors and Medical Panel Symposium, 2006; Tolk, 2006)).

The RTO had a Research and Technology Council, a technical section and technical teams. It was supported in its work by the RTA. About 140 research works were carried out annually in the technical teams. The most important body in the RTO was the Research and Technology Board (RTB). Its purpose was to direct and/or coordinate defence research and technology. Its board consisted of three defence research and technology specialists from each NATO country (NATO Research and Technology Organisation, 2017). The purpose of RTB was to conduct and promote joint research and information exchange. The task was to promote the development and effective use of national defence research and technology to meet the military needs of the Alliance, maintain technological superiority and provide advice to NATO. The RTO carried out its mission with the support of an extensive network of member country experts. It also ensured effective coordination with other NATO bodies involved in defence technology-related scientific and research activities (Curtis et al., 2013).

The research used to carry out the RTO's tasks was:

- Developing and maintaining a coordinated long-term strategy for NATO defence research and technology,
- Ensuring coordination and harmonization of work programs within NATO structures,
- Coordinating research and technology programs and activities between Alliance countries, as well as within NATO,
- Providing advice on research and technology issues to NATO's higher bodies,
- Financing joint studies and research projects,
- Conducting and promoting joint research activities, including tests and practical work,
- Promoting and facilitating the exchange of information on research and technology among NATO member states,
- Providing assistance to member states to enhance their scientific and technological capabilities.

These tasks were carried out in sections created for specific issues for a specific period of time. They were staffed by the most prominent scientists from member countries. Workshops, symposia, field trials, lecture series and training courses were organized. Their important function was to ensure the continuity of specialists, in addition to formulating long-term plans and research. Cooperation with Central and Eastern European countries was undertaken within the framework of the Partnership for Peace program. The RTO attached particular importance to this activity, as research cooperation was one of the more promising areas for initial cooperation (Curtis et al., 2013).

The RTA was the executive arm of the RTO, organizing a wide range of research, workshops and symposia through which scientists met and exchanged knowledge. The RTA had a rotating staff of about 30 civilian employees and 20 military personnel from member countries (NATO Research & Technology Organization Publications: NATO Collection, 2012).Its activities were carried out by six technical sections covering a broad spectrum of scientific and research activities:

- Vehicle Technology Panel (AVT),
- Human Factors and Medicine (HFM) Panel,
- Information Technology Panel (IST),
- Systems Analysis and Studies Panel (SAS),
- Systems Concepts and Integration Panel (SCI),
- Sensors and Electronics Technology Panel (SET),
- NATO Modelling and Simulation Group (NMSG),
- Information Management Committee (IMC)(NATO Structures: Research & Technology Organisation (RTO, 1997).

During its existence, the RTO was the sole centre of NATO's defence research and technology activities. Its mission was to promote and conduct joint research and information exchange. The goal was to promote the development and effective use of national defence research and technology to meet the military needs of the Alliance, maintain technological superiority and provide advice to decision-makers from NATO and Allied countries.

d) STO

The NATO Science & Technology Organization (STO) has been in operation since July 1, 2012, continuing the achievements of AGARD (1952-1996), DRG (1967-1996) and RTO (1998-2012). It was established to meet the scientific and technological needs of the Alliance. Its mission is to generate, share and disseminate advanced scientific knowledge, technological advances and innovations resulting from activities under the Collaborative Program of Work (CPoW). The STO acts as a forum through which representatives of Alliance member and partner countries have the opportunity to jointly define research needs, conduct research and promote its results, exchange knowledge, experience and information.

The executive bodies of the STO are:

- Office of the Chief Scientist (OCS), supporting the NATO Chief Scientist in his role as Chairman of the Science and Technology Board (STB) and Senior

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Scientific Advisor to NATO Senior Management (Science & Technology Board and Office of the Chief Scientist, 2013).

- Collaboration Support Office (CSO) providing executive and administrative support for activities under the collaborative business model (Collaboration Support Office (CSO), 2023).
- The Centre for Maritime Research and Experimentation (CMRE), which organizes and conducts scientific research and technology development, providing innovative and tested scientific and technical solutions to meet the Alliance's defence and security needs. Its tasks focus on maritime affairs, but can extrapolate to other domains to meet current needs (About the NATO Science & Technology Organization, 2015).

The STB's responsibilities include:

- Providing strategic guidance for science and technology in NATO by issuing the NATO S&T (Science & Technolgy) Strategy and NATO S&T Priorities.
- Directing and leading the STO Work Program.
- Providing science and technology advice in NATO decision-making processes.

There are seven sections within the STO:

- Applied Vehicle Technology (AVT).
- Human Factors and Medicine (HFM).
- Information Systems Technology (IST).
- Systems Analysis (System Analysis & Studies, SAS).
- Systems concepts and integration (Systems Concepts & Integration, SCI).
- Electronics and sensors (Sensors & Electronics Technology, SET).
- Modelling and Simulation (NATO Modelling and Simulation Group, NMSG). (Pages - Panel/Group Page, 2015).

Each year, more than 6,000 scientists and engineers from NATO and partner countries work on some 300 research projects. This results in the publication of highly regarded scientific literature published by STO: Technical Reports (TR), Educational Notes (EN) and Meeting Proceedings (MP). Research results are also published in specialized peer-reviewed journals (Nato.int., Collaboration Support Office (CSO), 2015).

e) NM&S

Within the STO is the NATO Modelling and Simulation Group (NM&S) (Mscoe.org., 2023). In November 1996, the Conference of National Armaments Directors (CNAD) established the NATO Simulation Policy and Applications Steering Group to develop the Alliance's approach to simulation for improving operations (including defence planning, training, exercises, support). CNAD recommended identifying recommended technical standards to support interoperability and use of simulation through the Modelling &Simulation Master Plan (MSMP). CNAD and the Military Committee (MC) approved it in November 1998 Two organizational structures were established: The NATO Modelling & Simulation Group (NMSG) and the Modelling & Simulation Co-ordination Office (MSCO) providing scientific, executive and administrative support to the NMSG (AGARD, RTO and STO History, 2020). The purpose of the MSMP is to promote cooperation among Alliance bodies, its member states and partner countries to maximize the effective use of modelling and simulation (M&S). The institution has three permanent subgroups:

- Military Operational Requirements Subgroup (M&S Subgroup).
- M&S Standards Subgroup (M&S Standards Subgroup).
- Planning and Programs Committee (Planning and Programs Committee) (Mscoe.org., 2023).

f) SPS

In 1958, the NATO Science Program was implemented to promote the training of scientists in Alliance countries and the position of Scientific Advisor to the NATO Secretary was created. On November 6, 1969, the Committee on the Challenges of Modern Society (CCMS) was established to combine research conducted in the various NATO countries and to create a common base for sharing experiences. Alliance members were increasingly aware of widespread environmental problems that could threaten the well-being and progress of their societies. There was already serious concern about the state of the environment and its degradation due to civilization. The potential offered by the development of technology and the possibility of its application for environmental protection was recognized. In the first decades of the Committee's activity, some 1,500 projects were funded, resulting from the cooperation of more than 6,000 scientists from Alliance countries. 650 scientific books and several thousand peer-reviewed scientific articles were published. More than 60,000 scholars took part in NATO-funded projects of so-called "Advanced Research," 12,000 were awarded NATO scientific fellowships (Science for Peace and Security (SPS) Programme, 2020). Scientific and research cooperation has also been established with the countries of the Mediterranean Dialogue (SPS News. 50 years. 1958-2008, 2008).

On January 1, 2003, the Committee received new regulations, the NATO Science and Environmental Protection Division was liquidated, and the Science Committee and its program were transferred to the newly established Public Diplomacy Division (PDD). On

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January 1, 2004, the NATO Program for Security Through Science was established. Research for safety has become a priority. In 2006, after the merger of the Scientific Committee and the Committee on the Challenges of Contemporary Society, the SPS (NATO Committee on Science for Peace and Security) was established.20 On November 1, 2010 (SPS News. N. 75 (3), 2006-2007). SPS was transferred from the PDD Division to the Emerging Security Division Challenges Division, ESCD) (New NATO division to deal with Emerging Security Challenges, 2020).

The SPS promotes dialogue and practical cooperation between NATO member states and partner countries based on scientific research, technological innovation and knowledge exchange. It offers financing, expert advice and support for tailored security activities that meet NATO's strategic objectives. It connects the scientific community with NATO through collaborative science that addresses emerging security challenges. Through SPS activities, researchers, scientists and experts play an important role in helping the Alliance identify, understand and respond to vulnerabilities and threats (Science for Peace and Security, 2020).Research includes: counterterrorism, energy security, cyber defence, defence against chemical, biological, radiological and nuclear (CBRN) agents and environmental security. The aim is to increase support for NATO-led operations and missions and increase awareness of security developments, including through early warning, to prevent crises (SPS - Key Priorities, 2020).

In 2013, the program was revised to focus SPS on larger-scale strategic activities beyond purely scientific cooperation. The SPS program is managed by NATO's Political and Partnerships Committee, which includes representatives of the countries involved in the cooperation. The evaluation of proposals submitted to the SPS program is carried out by a NATO body established for this purpose (Independent Scientific Evaluation Group, ISEG).

Since its establishment, STO has continued NATO's policy of conducting scientific and research work. An analysis of its activities allows us to conclude that its activities aim to best meet the collective needs of the Alliance and partner countries in science and technology. It conducts its activities by generating, sharing and disseminating advanced scientific knowledge, technological developments and innovations resulting from the many activities carried out in the security and environmental fields. It provides information and technology to meet the needs of the Alliance in an ever-changing security environment. What is noteworthy is that at present STO brings together the world's largest security and defence research network. It brings together scientists from member countries, engineers and analysts, industry and academia. STO's activities ensure that NATO maintains its military and technological edge to meet current and future security challenges.

g) Maritime Scientific and Research Institutions

The purpose of NATO's maritime scientific and research institutions was and is to continuously improve the efficiency of the operational activities of the Alliance's naval forces, test and implement innovative technologies, develop international cooperation in the scientific sphere of the Alliance member states, and protect the marine environment.

IV. Saclantcen

On May 2, 1959, the SACLANT ASW Research Centre (SACLANT AWS Research Centre, SACLANTCEN) was established. On October 20, 1962, the Centre came under the direction of the Supreme Allied Commander Atlantic (SACLANT). The scientific council that advised SACLANT in the early years evolved into the Scientific Committee of National Representatives (SCNR).

Initially, the scientific program mainly covered underwater acoustics, oceanography, evaluation of submarine warfare systems concepts and methods. Scientific groups were organized according to research criteria. The Underwater Acoustic Research Group conducted theoretical analysis, computer modelling and experiments at sea. The Oceanographic Research Group (The Oceanographic Research Group) built on this work by studying the marine environment and the interactions between the atmosphere and the sea. The groups were supported by the Technical Support Department. It carried out digital calculations, engaged in electronic and acoustic engineering. The research ships "Aragonnese", "Maria Paolina G." and "Manning" were used.

Until the mid-1970s, the Centre's research focused mainly on the open seas, a potential battlefield against Soviet submarine forces. Research in these bodies of water resulted in a number of scientific papers and implementations of new technologies. Some were among the innovative, including work on electromagnetic and surface effects at extremely low frequencies. There was a focus on acoustics and the use of sonar. Spatial frequency interference patterns of continuous waves, Frequency modulation (FM) techniques of sonar were developed. FM sonar was the most successful, and digital FM technique is still part of active sonar. The Reliable Acoustic Path project resulted in the construction of a "Deep Panoramic Sonar" based on a multiple array system known as MEDUSA (Mediterranean Experimental Deep Underwater Sonar Apparatus). This was the first active sonar developed at SACLANTCEN, and was used in experiments until 1973. In the mid-1970s, SACLANTCEN pioneered the use of underwater links to hydrophone buoys, resulting in a

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significant increase in data recording efficiency (Twenty Years Of Research At The Saclant, 1979).

In 1975, SACLANTCEN's scientific division was reorganized into two main divisions: the Environmental and Systems Research Division and the Operational and Analytical Research Division. The former had four working groups: deep-sea research, shallow water research, applied oceanography and signal processing, while the latter had three: effectiveness research, tactical research and theoretical studies. The focus was on littoral water research, oceanography and acoustics. In the late 1970s, work began on towed arrays, and testing of the first experimental linear hydrophone array began.

In 1988, a state-of-the-art NRV "Alliance" research and development unit with very low noise levels was put into service. To date, it is considered one of the quietest in the world. It spends an average of 170 days a year at sea on scientific research missions. Research on submarine detection and reconnaissance continued. In the early 1980s, work began on Low Frequency Activated Sonar (LFAS), from the Active Adjunct Project, using a passive towed sonar array and a high-power, low- to mid-frequency emitter. The goal of the Deployable Undersea Program Surveillance Systems (DUSS) program was to develop a static system for use in shallow waters. The project began in the early 1990s with conceptual studies, followed by initial sonar system development and at-sea testing. Improvements to the prototype resulted in DEMUS (Deployable Experimental Multistate Undersea System), delivered 2003.

In 1999, the Sound Ocean Living Marine Resources (SOLMAR) program was launched. This was a multinational, multidisciplinary project aimed at developing tools and/or procedures with which to ensure that there are no marine mammals in the vicinity of the sonar before and during its use. To achieve the research plans, a series of SIRENA sea trials were conducted between 1999 and 2003. Oceanographic measurements were conducted, including temperature, salinity, nutrients, fluorescence and phytoplankton. Sea surface temperature, surface currents and real-time wave action were also studied using satellite remote sensing (Ryan, 2008, 39). In 2002 SOLMAR was transformed into the Marine Mammal Risk Mitigation (MMRM) project. The passive acoustic sonars implemented in the instrument suite were the result of evolving methods and technologies used in subsequent research cruises under the SIRENA program. In addition, the impact of sonar on human factors was studied in all programs, including the work of divers (NATO Undersea Research Centre, 2006-2008, 1-21.

In 1986, a five-year survey of the Greenland, Iceland and Norwegian Seas (GIN) began. Advanced high seas Military Oceanography (MILOC) surveys continued. The goal was to detect and combat submarines and protect maritime communication routes. New technologies were implemented to improve sonar and underwater detection systems. With MILOC, oceanographic and acoustic databases supporting modelling were created, resulting in better use of operational sensors.

On November 16, 2009, the coastal CRV "Leonardo" was put into service. The 29-meter-long vessel is equipped with a variety of scientific and on-board facilities, in addition to having a very quiet propulsion system.

The number of scientists at SACLANTCEN, employed on temporary contracts, was up to 50. They were supported by administrative and technical teams, mainly the Engineering Department (ED), which provided the means to conduct experiments to develop or verify scientific theories. The Centre had a facility unique in Europe, the Oceanography Calibration Laboratory, which had been in operation since the early 1980s, supporting the activities of SACLANTCEN and NATO's naval and research laboratories. The Centre's scientific output was particularly valuable to member countries with less scientific and research capacity, helping to bridge the gap between their institutions and scientists in the US and UK. The organization was transformed into NURC in 2012.

An analysis of source materials shows that the establishment of SACLANTCEN was the result of the Cold War situation that had prevailed in the world since the 1950s. It was well realized that the Alliance's maritime security was of utmost importance. The main threat was Soviet submarine forces. In the first period of its activity, the organization's scientific program focused mainly on problems related to underwater acoustics, oceanography, evaluation of the concept of anti-submarine warfare systems and methods. Significant successes were achieved in the field of research and application of modern sonar. In the mid-1970s, moreover, SACLANTCEN pioneered the use of underwater links to hydrophone buoys. In 1988, the modern NRV research vessel "Alliance" was put into service, and in 2009, another for offshore research, "Leonardo." Particularly noteworthy is the launch of work on marine environmental protection, including the SIRENA program.

a) NURC

The NATO Undersea Research Centre (NURC) conducted world-class maritime research to support NATO's operational and transformational requirements. Emphasis was placed on the underwater area and solving maritime security problems. Subjects included research in underwater acoustic phenomena and their application to surveillance, detection, oceanography and port security (Barbagelata, Guerrini, Troiano, 2008, 24-33).

NURC's notable achievements include the development of a rapid detection algorithm for autonomous mine countermeasures, the

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implementation of a real-time multistate ASW simulation centre, a performance indicator for next-generation underwater surveillance networks, and numerical modelling of wave-current interactions at sea. NURC has a broad set of equipment for conducting experiments at sea: a fleet of AUVs (Autonomous Underwater Vehicle), ROVs (Remotely Operated Vehicle) and seabed survey platforms. NURC has developed research programs whose technologies have been adapted to underwater monitoring. Several projects used for military and civilian purposes have been developed, including the design and implementation of advanced environmental monitoring systems (BARNY, SEEP, SEPTR), which are used by many scientific centres around the world conducting oceanographic research.

The continuation and development of SACLANTCEN's research became NURC. The cited text shows that the previous scientific research work was continued and enriched with modern underwater warfare technologies and, importantly, continued to address issues related to monitoring of the marine environment. It should be noted here that the latter work is also used by civilian institutions.

b) CMRE

The Centre for Maritime Research and Experiments (Centre for Maritime Research and Experiments, CMRE) was established by the North Atlantic Council (NAC) on July 1, 2012, becoming the successor to NURC. It is the hardware organ of STO. CMRE's task is to organize scientific research and development of maritime-related technologies, providing innovative and field-proven technologies to meet the defence and security needs of the Alliance.

CMRE conducts cutting-edge research and experiments from concept development to prototype demonstration in a maritime operational environment. The centre employs specialists in, among others: oceanography, modelling and simulation, and acoustics. It has experienced engineering staff (ED) ensuring quick implementation of conceptual prototypes for trials and experiments. The centre has developed and tested at sea a large number of naval prototypes for anti-submarine warfare, mine countermeasures, maritime and port security and environmental monitoring.

CMRE provides advisory support on maritime defence and security issues to allied countries. It is equipped with a fleet of AUVs and a world-class marine sensor system. It is conducting deep advanced work on multi-static active sonar for searching and tracking a new generation of silent submarines (CMRE - Littoral ISR, 2023). The research project was also "Maritime Security-Port and Ship Protection"(CMRE - Port & Ship Protection, 2020).In turn, the Maritime Surveillance System (Maritime Situational Awareness, MSA) uses information from the Automatic Identification System (AIS). It applies anomaly detection algorithms and filters to provide NATO maritime surveillance forces with information about unusual situations, such as unexpected ship stops or radical course changes (CMRE - Maritime Situational Awareness, 2020). CMRE develops an annual research and development plan. His current scope of work includes research problems such as artificial intelligence, big data analytics, underwater acoustics, oceanography and autonomous systems (Science and Technology Organization Centre for Maritime Research and Experimentation, 2020).

In 2012, CMRE was established to replace NURC. As can be seen from the presented material, since its establishment it has been one of the world's leading institutions dealing with issues related to the development of maritime research and technologies. Scientific and research work is carried out in nine thematic areas and brings together the most outstanding scientists from the Alliance member countries.

V. Conclusion

The presented material, based on official NATO documents, shows the development of the Alliance's scientific and research institutions. They have played an extremely important role in strengthening NATO's defence potential and contributed to the significant development of technology and international cooperation. It should also be emphasized that thanks to them, the Alliance has become one of the leaders in programs and implementations related to environmental security.

Throughout its history, NATO has always attached great importance to conducting scientific and research work. S&T activities include scientific research, technology development, testing, field applications, experiments and a range of related scientific activities including systems engineering, operational research and analysis, synthesis, integration and validation of knowledge obtained through the scientific method. Thanks to NATO's policy, its scientific and research agendas are considered world-leading. It should be emphasized once again that the development of NATO's scientific and research institutions has made a significant contribution to strengthening the Alliance's defence and ensuring international security.

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Career | Credibility | Reputation

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Career | Credibility | Financial

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Exclusive | Financial

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Certificate, LoR and Momento **2** discounted publishing/year **Gradation** of Research **10** research contacts/day **1 GB** Cloud Storage **GJ** Community Access	**Certificate,** LoR and Momento **Unlimited** discounted publishing/year **Gradation** of Research **Unlimited** research contacts/day **5 GB** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**Certificates,** LoRs and Momentos **Unlimited** free publishing/year **Gradation** of Research **Unlimited** research contacts/day **Unlimited** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**GJ** Community Access

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Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

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The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

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The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

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It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

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Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

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A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

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Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

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Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

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Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

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7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

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This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

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Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
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Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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