**Task 5 for obtaining credits for B130P16E**

**(Practical basics of scientific work)**

**Task:**

Write a short manuscript of the scientific article with following title:

**"Carrier-driven auxin transport drives plant phototropic reactions"**

**Structure of the manuscript:**

**First page:**

Title, authors, affiliations – use your imagination

**Abstract**:

Try to briefly summarize the introduction into the topic, description of methods and results achieved and summarization of the relevance of the message that could be taken form this report (2-4 sentences).

**Key words**:

Find the most relevant terms according to which readers will find our article in the database

**Introduction:**

Put the reader into the true picture of these days knowledge in the field of auxin research. Mention briefly what is auxin and how important is its transport. The information could be find in the attached file (info\_auxin\_for\_task5.ppt).

**Material and methods:**

Describe the design of the experiments, you can find technical details below, do it briefly.

**Results:**

Assemble a consistent text describing results of your experiments, use appropriate subheadings, and refer to the respective image.

**Discussion:**

Discuss your results in the context of general knowledge and also try to make your own interpretations. Suggest some additional experiments if needed.

**Acknowledgments:**

Here you can specify grant support as well as mention colleagues or donators of the experimental material.

**Reference list:**

Make the reference list for all the papers that are mentioned in your article, they can be found in the file (info\_auxin\_for\_task5.ppt). Use Pubmed, WOS, or Scopus and Mendeley.

**Figure captions:**

A concise description of what is presented in the respective image. It should be self-explaining and easy to understand.

**Figures:** Always 1 figure per one page.

**Here is the explanation of two sets of experiments that you performed in the laboratory, including their description, rationale, data obtained, and techniques used:**

**Experiment 1:**

**Aim:**

To determine whether plant hormone auxin could, in theory, enter the cell by carrier-mediated transport.

**Background, description of the experiment and rationale:**

Two synthetic auxins, 2,4-D and NAA, enter the plant cell through the plasma membrane by two different mechanisms. The first utilizes specific membrane carrier (energy-dependent); the second is diffusion-based transport across plasma membrane.

The accumulation of radioactively-labelled auxins [3H]2,4-D and [3H]NAA inside 2-day-old tobacco cells BY-2 (Bright Yellow) was measured. For the assay, cells were transferred to the buffer of pH 5.8. After 2h of the equilibration in this buffer, radioactively labeled auxin ([3H]2,4-D or [3H]NAA) was added, and during a subsequent 30 min period, sampling of small aliquots was performed and the radioactivity determined in them using a scintillation counter. Control cells and cells treated with the inhibitor of the active transport inside cells 1-NOA (1-naphtoxyacetic acid) were used. An inhibitor was added 1 minute after the radioactivity.

The following values of radioactivity inside cells were obtained (data could also be downloaded as an excel file from the web page):

**For 2,4-D:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time (min) | Sample number | [3H]2,4-D (dpm) |  | [3H]2,4-D plus 1-NOA (dpm) |
| 0 | 1 | 4063 |
| 2 | 4635 |
| 3 | 5008 |
| 4 | 5309 |
| 3 | 5 | 13830 | 29 | 9881 |
| 6 | 14303 | 30 | 10046 |
| 7 | 12719 | 31 | 9792 |
| 8 | 14703 | 32 | 9715 |
| 5 | 9 | 16039 | 33 | 9175 |
| 10 | 15901 | 34 | 9389 |
| 11 | 15757 | 35 | 8727 |
| 12 | 16555 | 36 | 10175 |
| 11 | 13 | 16112 | 37 | 8722 |
| 14 | 16750 | 38 | 8486 |
| 15 | 16574 | 39 | 8787 |
| 16 | 16799 | 40 | 8834 |
| 17 | 17 | 14524 | 41 | 9106 |
| 18 | 15943 | 42 | 10500 |
| 19 | 16290 | 43 | 8674 |
| 20 | 16694 | 44 | 8843 |
| 25 | 21 | 15952 | 45 | 8128 |
| 22 | 15438 | 46 | 8509 |
| 23 | 15519 | 47 | 8444 |
| 24 | 16738 | 48 | 8744 |
| 32 | 25 | 15688 | 49 | 8357 |
| 26 | 15763 | 50 | 8789 |
| 27 | 15593 | 51 | 8281 |
| 28 | 16029 | 52 | 8278 |

**For NAA:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time (min) | Sample number | [3H]NAA (dpm) |  | [3H]NAA plus 1-NOA |
| 0 | 1 | 4827 |
| 2 | 5363 |
| 3 | 5586 |
| 4 | 5883 |
| 3 | 5 | 7360 | 29 | 7591 |
| 6 | 8076 | 30 | 8064 |
| 7 | 7898 | 31 | 8211 |
| 8 | 8269 | 32 | 8348 |
| 5 | 9 | 7164 | 33 | 7904 |
| 10 | 7446 | 34 | 7994 |
| 11 | 7822 | 35 | 8380 |
| 12 | 7260 | 36 | 8461 |
| 11 | 13 | 8187 | 37 | 8304 |
| 14 | 7279 | 38 | 8505 |
| 15 | 7682 | 39 | 8113 |
| 16 | 7734 | 40 | 7987 |
| 17 | 17 | 7308 | 41 | 8040 |
| 18 | 7432 | 42 | 8134 |
| 19 | 8279 | 43 | 8446 |
| 20 | 7765 | 44 | 8168 |
| 25 | 21 | 7669 | 45 | 8192 |
| 22 | 7516 | 46 | 8198 |
| 23 | 7416 | 47 | 8376 |
| 24 | 7812 | 48 | 8588 |
| 32 | 25 | 7710 | 49 | 8316 |
| 26 | 8581 | 50 | 8610 |
| 27 | 8301 | 51 | 8860 |
| 28 | 8064 | 52 | 8931 |

Make plots from these values representing how the radioactive auxin accumulates inside cells. Based on the data, try to conclude which of these two auxins enter the cell passively and which actively. Results of this experiment also clearly show that at least one of these two synthetic auxins enters plant cells by active carrier-mediated transport across the plasma membrane.

**Experiment 2:**

**Aim:**

To determine whether carrier-mediated transport could have some role in the bending reactions of plants towards light.

**Background, description of the experiment and rationale:**

This experiment tested the importance of carrier-mediated transport in planta. We were interested in what would be the consequence of the inhibition of active auxin efflux carrier for the reaction of plants to the light (phototropism).



From this image (take it as the result of your work for the purpose of this trial manuscript) it is obvious that the inhibitor of auxin efflux carrier NPA (1-N-naphtylphtalamic acid) disturbed the reaction of *Arabidopsis thaliana* seedlings stems on the directional illumination from the right side. Under standard conditions **(a)** the stem is bending towards the light. After NPA treatment **(b)** the stem is not bending at all. This image is also demonstrating how the auxin is distributed in this plant. The blue color indicates sites where the gene expression provoked by auxin takes place. Clearly, it is enhanced at the shaded side of the stem, which is elongating more than the illuminated side. After the application of NPA, this gradient cannot be formed. We ask the question on the importance of this process for the elongation of cells at both sides of the stem.

For the purpose of this experiment, plants were transformed with the gene coding for ß-glucuronidase enzyme under the promoter sensitive to auxin. The expression of this enzyme takes place only when there is a sufficient amount of auxin around. Its activity subsequently creates a blue precipitate after the addition of chromogenic substrate to the fixed plants, X-gluc (5-bromo-4-chloro-3-indolyl ß – D glucuronide).

From this experiment, you can conclude (at least indirectly), what is the role of active auxin transport during the bending of the stem of A*rabidopsis thaliana* seedling. Describe the experiment in your words.