## THE SECRETS OF FIRE ANT COMMUNICATION: FOCUS ON PBAN



### Interaction between PBAN and its receptor

#### Context

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In the context of chemical communication in insects, where pheromones play a vital role, the endocrine regulation of their production is a complex and little-studied area, particularly in fire ants. The researchers therefore turned to the PBAN peptide, known for its role in pheromone biosynthesis in Lepidoptera, with the aim of extending our knowledge of trail pheromones in fire ants. In this context, the protagonists are fire ants, the neuropeptide PBAN and its receptor R-PBAN, and trailing pheromones. This choice of study is based on the poorly developed knowledge of how fire ants follow each other when one of them finds a resource they need. What's more, these protagonists have already been discovered as activators of sex pheromone biosynthesis in moths. The results obtained demonstrated that PBAN effectively regulates pheromone biosynthesis in these insects, opening up new avenues of research into the endocrine regulation of pheromones in different insect species. The aim is to see if PBAN interacts with its receptor, thereby influencing the production of drag pheromones in these ants. To achieve this goal, the researchers used fluorescence and gene expression inhibition strategies targeting the PBAN gene or its receptor in fire ants.

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fluorescence

## SolinPBAN-R/peptide binding assays

The fluorescent probe Fluo-4AM is used here. It is a lipophilic, negatively charged calcium indicator capable of crossing cell membranes. Inside the cell, esterases cleave the AM part (methylimine acetate) of the probe, making it hydrophilic and trapped in the cells. In the presence of Ca2+, the Fluo-4 part binds to it and fluorescence increases.

Specifically, the PBAN receptor, when it interacts with its ligand PBAN, allows the entry of Ca2+ into the cell. Thus, the use of the Fluo-4 probe provides information about the interaction between PBAN and R-PBAN.

## Results

Log[peptide] (M)

It can be seen that the fluorescence curve for SolinPBAN increases much more rapidly with higher concentrations (EC50 = 18 nM) than the fluorescence curve for SolinDH (EC50 = 125 nM). This suggests that PBAN has a greater affinity for its receptor than other hormones, and that there is indeed an interaction between the PBAN-R receptor and SolinPBAN, its ligand.

#### Pros

- Direct technique
- Sensitive
- In real time

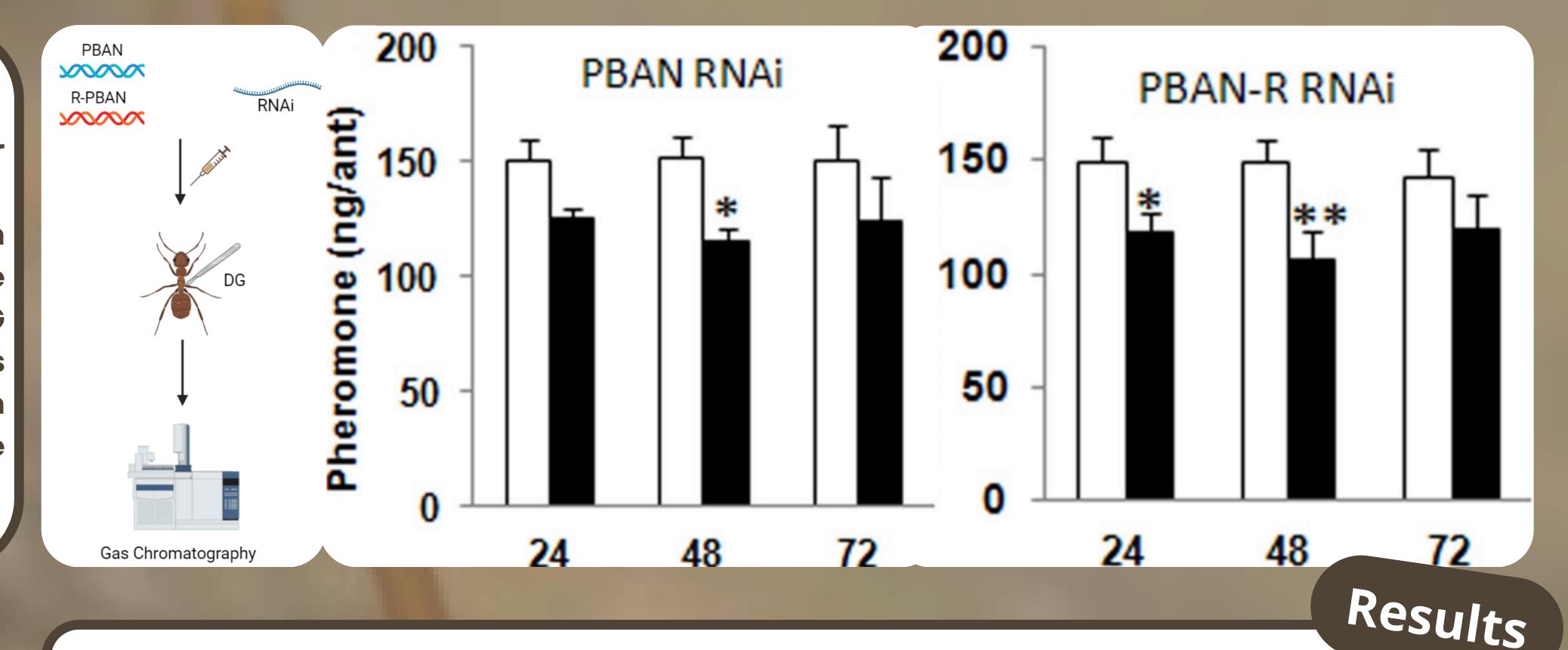
#### Cons

- Non-specificity
- Potential toxicity
- Complex handling

# RNAi suppression of SolinPBAN and SolinPBAN-R gene expression

It's a quantification of pheromone according to whether RNAi-PBAN or RNAi-RPBAN was used or not.

For that ants were injected with PBAN and PBAN-R, then PBAN RNAi or PBAN-R ARNi was added, and left to incubate at different times. The ants were then dissected at the DG level and the trail pheromones were quantified by gas chromatography. Pheromone analysis was compared with another reference compound. The results obtained are relative and were analyzed using statistical software.



#### Pros

- High sensitivity
- Accuratequantitation
- High resolution

#### Cons

- Volatility needed
- Detection limits
- Thermal degradation

Injection of SolinPBAN RNAi significantly inhibited track pheromine production at 48h. When PBAN-R RNAi was injected, it significantly inhibited track pheromone production at 24h and 48h. Pheromone production is only possible if SolinPBAN and PBAN-R interact with each other. We have therefore seen here that if one of these two protagonists is suppressed, production is inhibited. This clearly demonstrates inderectly the interaction between SolinPBAN and PBAN-R.

# conclusion

The neuropeptide SolinPBAN stimulates the biosynthesis of track pheromones in ants when it interacts with its PBAN-R receptor located in the Dufour gland region of the fire ant. This was demonstrated using fluorescence and gas chromatography. This interaction enables fire ants to acquire resources, maintain social structure and territoriality. Looking ahead, the use of other more direct techniques such as two-hybrid and immunoprecipitation may be used to demonstrate interaction between PBAN and its receptor. In addition, this project suggests new methods for controlling insect pests.