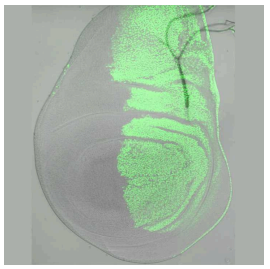
Sep 17, 2022

Auxin-induced (AID) protein degradation in drosophila larvae



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We use this protocol and it's working

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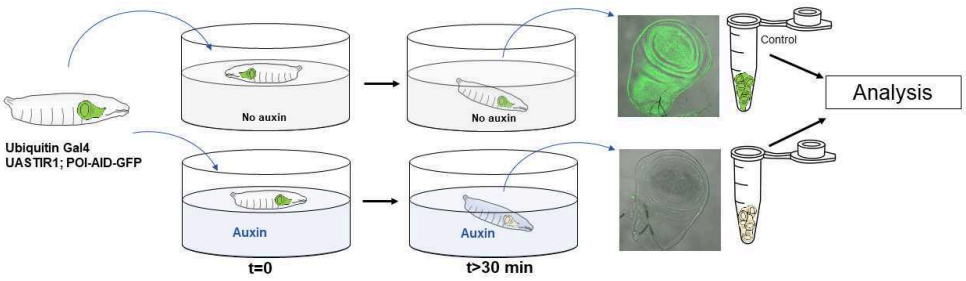
Abstract

The present protocol describes how to operate the auxin degradation system in drosophila larvae.The auxin-induced degradation, also referred to as AID, is an efficient targeted protein degradation system widely used in many model organisms. Thanks to the fact that fast degradation is triggered once a small molecule, the auxin, is added to the cell environment, a tight temporal control of the loss of a protein of interest can be achieved. This unique control of when the proteolysis is triggered allows to study the precocious consequences of the loss of function. Importantly, the implementation of this protocol requires genetically modified flies that express the auxin-dependent F-box protein TIR1, usually under the control of the UAS/Gal4 system to achieve a spatial control of the degradation, and in which the AID degron has been inserted (typically using CRISPR) to the coding sequence of the gene of interest. The problem raised by the use of the AID system in drosophila larvae, as in all the metazoans that have thick tegument, is the penetration of the auxin to each indidual cell of the organism. Here we present a non-invasive strategy based on the ingestion by the larva of a nutritive medium that contains auxin. We detail how to prepare the auxin containing food, handle the larvae, and which food container to use. Our method allows a fast degradation in the imaginal discs, as early as 30 minutes after the larvae were transfered to the auxin containing medium, with little inter-individual difference.



Schematic view of the protocol used to operate the AID system in drosophila larvae. Here, a genetic configuration where the expression of TIR1 is driven by the Ubiquitin promoter is shown.

Attachments

2022.06.06.494962.fu..

2MB

Guidelines

The method described here to induce targeted protein degradation with the AID system in drosophila larva is based on feeding the animal with auxin containing nutritive medium. Once present in the digestive asparatus, auxin penetrates in the tissues of the larva.

The protocol workflow is:

Day 1: young well-fed adults are transfered to fresh fly tubes.
Allow the flies to lay eggs for 15 hours, then remove the adults from the tube. This allow to get larvae all at a similar stage, and also avoid overcrowding.

Day 3: prepare auxin containing petri dishes
30 mm plastic petri dishes containing a nutritive medium that contains auxin need to prepared.

Day 4 to day 5: collect larvae and trigger protein degradation, and proceed to phenotype analysis.
To trigger protein degradation, larvae are transfered using forceps to the auxin dishes.

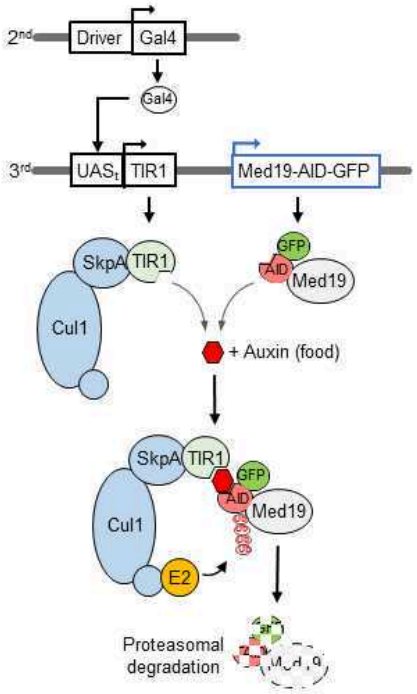
Materials

- Bacteriological agar **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A5306
- Sucrose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S9378
- 1-Naphthaleneacetic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**N0640

Equipment

Fine Forceps	NAME
Forceps	TYPE
Dumont	BRAND
11251-10	SKU
https://www.finescience.com/en-US/Products/Forceps-Hemostats/Dumont-Forceps/Dumont-5-Forceps/11251-10	LINK

- 30 mm plastic petri dishes
- Drosophila malanogaster flies with a genotype similar to what shown in the following figure.



Genetic and molecular set-up used for the AID-mediated degradation of Med19 in drosophila. The expression of the auxin-dependent and AID-specific F-box protein TIR1 is controlled by the UAS/Gal4 system. The Gal4 driver is located in the second chromosome, when the UAS::TIR1 is positioned to the third chromosome where the Med19* gene lies. The F-box TIR1 incorporates the endogenous SCF (Skip1 Cul1 F-box) E3 ubiquitin complex c. Auxin (added to the fly food) triggers poly-ubiquitination of Med19* by the SCFTIR1 targeting the Mediator subunit to degradation by the proteasome.

Protocol materials

- Bacteriological agar **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A5306
- Sucrose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S9378
- 1-Naphthaleneacetic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**N0640
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- 1-Naphthaleneacetic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**N0640
- Bacteriological agar **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A5306
- Parafilm M **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P7793

Troubleshooting

Safety warnings

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).
Auxin is toxic when used on cells above 50 mM.

Before start

Genetically modified flies need to be generated before the present protocol can be implemented.
These flies should contain:
1) a transgene allowing the expression of the auxin-dependent F-box protein TIR1, usually under the control of the UAS/Gal4 system (to achieve a spatial control of the degradation),
2) the AID degron inserted (typically using CRISPR) to the coding sequence of the gene of interest.
See materials for an illustrative figure.

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Carry out AID protein degradation in drosophila larvae

5d 10h 28m

1 Initiate larvae production

Note

In our hands, we found it is time saving to initially invest in generating genetic setups that are ready to use in term of induction of protein degradation by auxin (no crosses required to generate the larvae with the right genetic background allowing the AID system to work). As an example, in the case of degradation of Med19-AID (3rd chromosome), we recombined the UASt::TIR1 transgene (3rd Chromosome) with the Med19-AID allele, and use the 2nd chromosome to carry the Gal4 driver. For instance, for a degradation of Med19-AID directed in the posterior domain of the wing imaginal disc, the genotype for a ready to use system is enGal4; Med19-AID-GFP, UAStTIR1 (see materials for a figure).

1.1 Transfer in standard wheat cornmeal fly tubes about 50 healthy young adult flies (1/3 males, 2/3 females) per tube 30m

Note

See the fly genotype required at this step in the previous note. It is essential to perform a no degradation control. Proper fashions to do so include using an identical genotype except that: possibility 1) it is devoid of the gal4 driver, or possibility 2) the gene of interest is devoid of the AID degron.

1.2 Let the flies lay eggs Overnight at 25 °C 15h

1.3 Remove the flies from the tubes 30m

1.4 Incubate the emptied tubes for 96:00:00 at 25 °C until the wandering L3 Larvae appear 4d

2 Preparation of NAA containing medium 15h

Note

NAA (1-Naphthaleneacetic acid) is a synthetic analog of Auxin offering better solubility and stability.

2.1 Mix 1.2 g of agar Bacteriological agar **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A5306 , 8 g of sucrose Sucrose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S9378 , and 0.093 g of NAA 1-Naphthaleneacetic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**N0640 , with 200 mL of water. 30m

Note

The composition of the degradation medium is 0.6% agar, 4% sucrose, 2.5mM [M] 2.5*10^-3 Molarity (M) NAA, in water

If required, depending on the nature of the negative control, prepare the same mixture without NAA to produce the control medium. Note that in flies expressing TIR1, a leaking degradation of the AID-tagged protein occures in the absence of auxin.

2.2 Microwave until complete agar melting 5m

2.3 Cool down to 70 °C 5m

2.4 Pour 30mm plastic petri dishes (5ml of melted agar solution per dish) 20m

2.5 Store in a plastic bag at 4°C protected from light

Note

The agar NAA medium is stable for two weeks when properly stored.

3 Triggering protein degradation in L3 larvae 30m

3.1 Equilibrate NAA 30 mm dishes, and control dishes, prepared as described in section 2, to room temperature, and add a pinch of yeast granulates to the dishes 30m

3.2 Using forceps, gently but quickly transfer up to 50 L3 larvae from tubes prepared as described in section 1 to a 30 mm dish containing NAA (same for control no NAA agar dish) 15m

3.3 Quickly seal the dishes using a piece of parafilm 2m

Parafilm M **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P7793

to prevent the larvae from escaping

3.4 Puncture tiny holes (small enough so that larvae cannot pass through) in the parafilm membrane using a needle in order to ensure oxygen and CO2 exchange 5m

3.5 Incubate the dishes at 25 °C the required amount of time 1h

Note

Pronounced degradation is usually achieved within 30 minutes.

3.6 Remove the parafilm seal 1m

3.7 Using forceps, gently collect the larvae from the agar dishes, and quickly proceed to desired operation, typically dissection of the imaginal discs. 5m