Agència
de Gestió d'Ajuts
Universitaris
i de Recerca

Memòria justificativa de recerca de les convocatòries BCC, BE, BP, CTP-AIRE, DEBEQ, FI, FI-ICIP, INEFC i PIV

La memòria justificativa consta de les dues parts que venen a continuació:
1.- Dades bàsiques i resums
2.- Memòria del treball (informe científic)

Tots els camps són obligatoris

1.- Dades bàsiques i resums

Nom de la convocatòria
BP

Llegenda per a les convocatòries:

BCC	Convocatòria de beques per a joves membres de comunitats catalanes a l'exterior
BDH	Beques i ajuts postdoctorals del Programa DGR-Henkel KGaA
BE	Beques per a estades per a la recerca fora de Catalunya
BP	Convocatòria d'ajuts postdoctorals dins del programa Beatriu de Pinós
CTP-AIRE	Ajuts per accions de cooperació en el marc de la comunitat de treball dels Pirineus. Ajuts de mobilitat de personal investigador.
DEBEQ (Modalitat A3)	Beques de Cooperació Internacional i Desenvolupament
FI	Beques predoctorals per a la formació de personal investigador
FI-ICIP	Beques i ajuts per a l'etapa de formació i de recerca de personal investigador novell en els àmbits d'interès de l'Institut Català Internacional per la Pau
INEFC	Beques predoctorals i de col·laboració, dins de l'àmbit de l'educació física i l'esport i les ciències aplicades a l'esport
PIV	Beques de recerca per a professors i investigadors visitants a Catalunya

Títol del projecte: ha de sintetitzar la temàtica científica del vostre document.
Bristle pattern formation in the thorax of Drosophila: a systems-level approach

Dades de l'investigador o beneficiari

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Número d'expedient
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Paraules clau: cal que esmenteu cinc conceptes que defineixin el contingut de la vostra memòria.
modelling
gene networks
pattern formation
quantitative data analysis
Drosophila wing disc

Data de presentació de la justificació
26 Setembre 2011

Nom i cognoms i signatura
del/de la investigador/a

BÀRBARA NEGLE de BOFARULL

Vist i plau del/de la responsable de la
sol·licitud

JOHANNES JAEGER

Generalitat de Catalunya
Departament d'Economia
i Coneixement

Resum del projecte: cal adjuntar dos resums del document, l'un en anglès i l'altre en la llengua del document, on s'esmenti la durada de l'acció

Resum en la llengua del projecte (màxim 300 paraules)

A fundamental question in developmental biology is how tissues are patterned to give rise to differentiated body structures with distinct morphologies. The Drosophila wing disc offers an accessible model to understand epithelial spatial patterning. It has been studied extensively using genetic and molecular approaches. Bristle patterns on the thorax, which arise from the medial part of the wing disc, are a classical model of pattern formation, dependent on a pre-pattern of trans-activators and -repressors. Despite decades of molecular studies, we still only know a subset of the factors that determine the pre-pattern.

We propose a novel and interdisciplinary approach to predict regulatory interactions in this system. It is based on the description of expression patterns by simple logical relations (addition, subtraction, intersection and union) between simple shapes (graphical primitives). We propose to perform a systematic analysis of gene expression patterns in the medial wing disc to identify such primitives. Similarities and relations between primitives have been shown to be predictive of regulatory relationships between the corresponding regulatory factors in other systems, such as the Drosophila egg. Furthermore, they provide the basis for dynamical models of the bristle-patterning network, which enable us to make even more detailed predictions on gene regulation and expression dynamics. Predicted regulatory interactions will then be tested by analysis of mutant phenotypes.

We expect to identify new regulatory interactions and to understand the basic dynamics of the regulatory network responsible for thorax patterning. These results will provide us with a better understanding of the rules governing gene regulatory networks in general, and provide the basis for future studies of the evolution of the thorax-patterning network in particular.

Resum en anglès (màxim 300 paraules)

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Resum en anglès (màxim 300 paraules) – continuació -.

2.- Memòria del treball (informe científic sense limitació de paraules). Pot incloure altres fitxers de qualsevol mena, no més grans de 10 MB cadascun d'ells.

Project outline

A fundamental question in developmental biology is how tissues are patterned to give rise to differentiated body structures with distinct morphologies. The Drosophila wing disc offers an accessible model to understand epithelial spatial patterning. It has been studied extensively using genetic and molecular approaches. Bristle patterns on the thorax, which arise from the medial part of the wing disc, are a classical model of pattern formation, dependent on a pre-pattern of trans-activators and -repressors. Despite decades of molecular studies, we still only know a subset of the factors that determine the pre-pattern.

In this project we proposed a novel and interdisciplinary approach to predict regulatory interactions in the thorax bristle patterning system of *Drosphila*. It is based on the description of expression patterns by simple logical relations (addition, subtraction, intersection and union) between simple shapes (graphical primitives). We proposed to perform a systematic analysis of gene expression patterns in the medial wing disc to identify such primitives. Similarities and relations between primitives have been shown to be predictive of regulatory relationships between the corresponding regulatory factors in other systems (Yakoby et al 2008). Furthermore, they provide the basis for dynamical models of the patterning network, which enable us to make even more detailed predictions on gene regulation and expression dynamics. Predicted regulatory interactions will then be tested by analysis of mutant phenotypes.

Research objectives

We proposed to use an interdisciplinary, systems-level approach to obtain new insights into the regulatory network responsible for thorax patterning. Our main objective is to predict novel interactions in the regulatory network responsible for patterning of the *Drosophila* thorax through the use of constructive solid geometry (Foley et al. 1995) and methods from dynamical systems theory (Strogatz, 2001, Hirsch et al. 2004).

We aim to:

- Identify groups of genes with similar expression patterns (by *in situ* hybridisation).
- Analyse these expression patterns in terms of combinations of simple shapes (primitives).
- Use the above analysis to predict the regulatory network responsible for thorax patterning.
- Test these predictions by functional perturbation assays in vivo.

Research methodology

This project has one main objective: to predict and verify novel regulatory interactions responsible for patterning the *Drosophila* thorax. We want to take a global or systems-level approach by looking at and classifying expression patterns of as many regulators and targets as possible with the ultimate aim of being able to explain the spatial expression patterns of the patterning genes *sr* (which determines the position of the muscle attachment sites) and *sc* (which determines bristle position). This goal can be subdivided into three main steps (data collection, modelling and validation) that needed to be performed in a consecutive manner.

1. *Characterization and classification of expression patterns in the prospective notum*
2. *Prediction and modelling of regulatory interactions*
3. *Verification of predicted regulatory interactions*

(A detailed description of the research methodology can be found in the annex of the BP application, a copy is provided with this report).

Progress and Updated Future Work plan

I have performed the first steps of the project mostly as originaly proposed. Only minor adjustments have been made to the original workplan.

I have been focusing on Aim 1 (see below). I have selected the candidate genes and standardised all the procedures for *in situ* hybridisation and data collection. I am now in the process of obtaining the expression patterns for all the candidate genes (patterns for 30 genes have been obtained so far).

I will briefly describe the steps wich have already been performed and then update on the steps that still need to be done. An updated Gantt Chart for the project is provided in Table 1 (see below).

- Aim 1: Characterization and classification of expression patterns in the prospective notum

Selection of candidate genes from the literature

I have obtained a list of candidate genes from the literature, the selected genes show at least one of the following criteria:

- Genes described to play a role in patterning the *Drosophila* thorax.
- Genes with enhanced expression in the thorax anlagen or the wing disc, as determined by microarray studies.

The list includes 313 genes from which we have chosen 175 candidates to perform *in situ* hybridiations. For the selection of these 175 genes we have focused on transcription factors and signalling molecules, and also included the most reliable candidates from other relevant categories. We have obtained clones for 159 of these genes from the Berkeley Drosophila Genome Project (BDGP), six from Pat Simpson's laboratory (University of Cambridge) and the remaining ten have been cloned by PCR.

Standardization of in situ *hybridizations and data acquisition*

We had planed to perform the *in situ* hybridisations on Drosophila imaginal discs with an *in situ* robot. This has not been possible due to technical problems. However I have set up a simplified protocol for manual *in situs*. It is more time consuming than a robot but gives much more consistent results.

I have also adapted the protocol to perform double *in situs* on imaginal discs. The gene *wingless (wg)* has shown to be a good marker for confirming the relative position of expression patterns.

A crucial step in the standardization has been to stablish procedures for mounting and data recording which allow for automatic recognition of wing disc shape and orientation. Briefly, discs are mounted in a slide separated enough to avoid having more than one disc in the same picture. Two pictures for each disc are taken simultaneously (to ensure they have exactly the same frame): one with DIC optics (which allows for the automatic detection of the wing margin), and a second one with Bright Field (from which the expression pattern of the gene is extracted – blue staining). Images of left discs are manually flipped to facilitate automatic processing and image comparison.

Obtain expression patterns by in situ *hybridization*

At present I am mainly working on *in situ* hybridisations and data recording:

- I am dissecting late third instar larvae (5-6 days old, 25C),
- fixing the imaginal discs for *in situ* hybridisation,
- obtaining RNA- DIG labelled probes from gene clones,
- performing enzymatic *in situ* hybridisations in wing discs with all the candidate genes,
- dissecting and mounting stained wing discs, and
- recording expression patterns (BF and DIC images).

We are adapting an existing processing pipeline for embryos (Surkova et al. 2008) for the analysis of the wing disc images and extraction of expression patterns. The more complex shape of the wing disc has made it necessary to add some processing steps to the pipeline. We are implementing the method of Ahammad et al (2005) which uses a method called 'congealing' to obtain an average disc shape and allows to adapt each individual image to the average shape in one step. Once each image is adapted to the average shape we can easily compare all the stainings from the same gene and obtain a canonical expression pattern for the gene. These canonical expression patterns will be the basis of the following analysis.

Analysis of expression patterns and selection of graphical primitives

The availability of canonical expression patterns on an average disc (see previous section) will make it possible to directly compare all expression patterns between them. To obtain suitable graphical primitives the obtained expression patterns will be analyzed as follows:

- Complex expression patterns will be subdivided by domains of expression (e.g. *sr* would be subdivided in 4 areas)
- Patterns will be classified using specific criteria, for example similarity, expression in anterior vs posterior and/or medial vs lateral regions.

Each group of patterns will be characterized by a shared shape or standard pattern. This set of standard patterns will be used to extract graphical primitives (see Figure 1 for an example). Based on this, we will:

- Compare shared pattern elements to see if some patterns can be explained by logical combinations of others. This allows us to identify some putative primitives and to reduce the set of patterns to explain.
- This first set of primitives will be the basis for finding other primitives which help explain the remaining patterns. This process will be repeated iteratively until an optimal set of primitives is found (the set with the minimum required number of basic shapes).

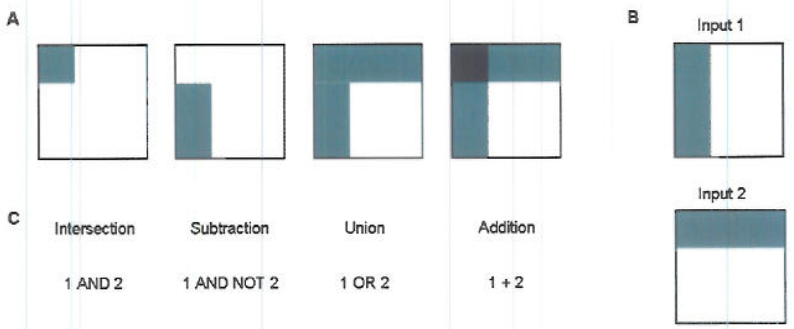

***Figure 1**. Examples of hypothetical graphical primitives and the geometrical operations that can be used to describe expression patterns. **(A)** Examples of expression patterns. **(B)** Graphical primitives suitable to describe expression patterns shown in **A**. **(C)** Geometrical operations on primitives that predict regulatory relationships between inputs required for the patterns shown in **A**. Modified from Yakoby et al. (2008).*

- Aim 2: Prediction and Modelling of Regulatory Interactions

To obtain dynamical network models we will:

- Analyze combinations of graphical primitives to derive logical relationships between factors.
- Compare predicted relationships with the existing experimental literature to identify novel and interesting predictions.
- Implement networks as an abstract, signed regulatory graph.

This graph will then provide the basis for the construction of logical and continuous models of specific aspects of the thorax-patterning network. Which aspects we will focus on, will be based on the

comparison between predicted relationships and the experimental literature. **Once this is achieved we will start Aim 3 (see below) and proceed in parallel with both Aim 2 and Aim3.**

Logical models will be implemented in the GINsim package (Naldi et al. 2009). The regulatory graph of the network is sufficient to define such a model. We will:

- Analyse the steady-states of the model to check whether they represent observed expression patterns.
- If they do, we will identify regulatory feedback mechanisms responsible for the observed expression features.

Continuous models will be implemented in Matlab (or an equivalent numerical package). The regulatory graph of the network only constrains parameter values of this model, but does not completely determine them. We will:

- Sample constrained parameter ranges to test whether the model can achieve robust patterning corresponding to observed expression patterns.
- If this is not the case, we will fit the model to data using non-linear optimization, to obtain parameter sets than can reproduce the observed patterns (cf. Jaeger et al. 2004a,b, Fomekong-Nanfack et al. 2007, Ashyraliyev et al. 2008).

Once we have obtained models that reproduce the observed patterns, we will numerically analyse them to describe the dynamical behaviour of the system. In particular, we will identify steady-states, and the regulatory mechanisms responsible for specific patterning features, similar to early studies using continuous network models (Jaeger et al. 2004a,b, Manu et al. 2009a,b).

- Aim 3: Verification of predictions

To check the reliability of our models we will chose a set of interactions based on network topology and the specific questions we want to address. We will functionally test these interactions by experimental perturbation of the system. Specifically, we will:

- Check the effect of mutant alleles of a given gene by clonal analysis.
- For those genes where mutants are not available we will knock down its expression by RNAi.

The clonal analysis will be done by site-specific recombination with the FLP/FRT system. We will:

- Drive recombination with Hsp70-promoted FLP constructs, which allow controlling the timing of recombination by heat-shock.
- Use GFP to mark the clones (or absence), to allow the identification of clones in the discs.

For RNAi knock-down experiments we will:

- Use the GAL4-driven RNAi transgenic lines developed at the Vienna Drosophila RNAi Stock Center (http://stockcenter.vdrc.at). If available, more than one line will be tested to verify the specificity of the knock-down effect.
- The expression of the RNAi constructs will be driven by GAL4 lines with expression in all or part of the thorax anlagen. E.g. ap-GAL4 (expressed in the dorsal compartment of the wing disc – covers the whole thorax anlagen), pnr-GAL4 (expressed in the medial half of the thorax),
- Verify the absence (or reduction) of the targeted gene by RT-PCR or *in situ* hybridization.

To check the effect these perturbations have in the network we will:

- Check the behaviour of reporter constructs. E.g. DC-lacZ which is expressed in a small area in the central region of the thorax anlagen.
- Check the behaviour of putative downstream genes by *in situ* hybridization.

The results of the experimental perturbations will be compared with the expectations form our models. In case of discrepancy we will introduce the new data into the models to improve their relevance, accuracy and predictive power.

Courses Attended

During this year I have also attended two courses that will contribute to the success of this project and to my future career prospects.

- *Practical summer course: Modelling for Systems Biology* (organised by the CRG-EMBL Systems Biology Unit). This one week course provided a broad introduction to systems biology, with a focus on dynamical modelling of networks. It included theoretical lectures during the morning covering the following topics: dynamical systems theory, stochastic systems, frameworks and methods to model biological networks, multivariate and multidimensional data analysis, techniques for parameter inference, reverse engineering, biophysical and cellular models. All theoretical parts of the course were also accompanied by hands-on exercises during the afternoons. In addition to introducing me to new techniques and know-how, this course was a great opportunity to interact with established figures in the field of systems biology.
- *How to write a grant proposal* (organised by the Intervals program of the PRBB). This two day course offered the tools to plan and write high-impact research proposals that tell a powerful story to the granting agency. It also gave an overview of the programs and granting agencies currently available in Spain and the EU. This course also gave me the opportunity to write and defend my own grant proposal, which was evaluated by a panel composed of two group leaders from the PRBB.

Table 1. Revised Gantt chart for the project.

	Year	1				2			
	3 month periods	1	2	3	4	1	2	3	4
Aim 1.	**Characterization of expression patterns**								
	Selection of candidate genes								
	Standarization of *in situs* and data recording								
	Perform *in situs* and data recording								
	Pattern analysis and selection of primitives								
Aim 2.	**Prediction and Modelling**								
	Modelling and network description								
	Feedback from experimental perturbations								
Aim 3.	**Verification of predictions**								
	Experimental design								
	Crosses, stainings and data recording								
	Analysis of results								
•	**Dissemination of results**								
	Unit talks and lab meetings								
	Conference communications								
	Manuscript(s) preparation								
•	**Supplementary skills**								
	Project management								
	Attendance of additional courses								
	Career development activities								

REFERENCES

Ahammad P., C.L. Harmon, A. Hammonds, S.S. Sastry and G.M. Rubin (2005) Joint nonparametric alignment for analyzing spatial expression patterns in Drosophila imaginal discs. CVRP (2) (IEEE Computer Society, Washington, DC, USA), pp.755-760.

Ashyraliyev M., J. Jaeger and J. Blom (2008) Parameter estimation and determinability analysis applied to *Drosophila* gap gene circuits. *BMC Syst Biol* **2**: 83.

Foley J.D., A. van Dam, S.K. Feiner and J.F. Hughes (1995) Computer Graphics: Principles and Practice (2nd Edition). Addison-Wesley, Reading, MA, USA.

Fomekong-Nanfack Y., J.A. Kaandorp and J. Blom (2007) Efficient parameter estimation for spatio-temporal models of pattern formation: case study of Drosophila melanogaster. *Bioinformatics* **23**: 3356-3363.

Hirsch M.W., S. Smale and R.L. Devaney (2004) Differential Equations, Dynamical Systems & An Introduction to Chaos (2nd Edition). Academic Press, London, UK.

Jaeger J., S. Surkova, M. Blagov, H. Janssens, D. Kosman, K.N. Kozlov, Manu, E. Myasnikova, C.E. Vanario-Alonso, M. Samsonova, D.H. Sharp and J. Reinitz (2004a). Dynamic control of positional information in the early *Drosophila* blastoderm. *Nature* **430**: 368–371.

Jaeger J., M. Blagov, D. Kosman, K.N. Kozlov, Manu, E. Myasnikova, S. Surkova, C.E. Vanario-Alonso, M. Samsonova, D.H. Sharp and Reinitz J. (2004b) Dynamical analysis of regulatory interactions in the gap gene system of *Drosophila melanogaster*. *Genetics* **167**: 1721–1737.

Manu, S. Surkova, A.V. Spirov, V.V. Gursky, H. Janssens, A.-R. Kim, O. Radulescu, C.E. Vanario-Alonso, D.H. Sharp, M. Samsonova and J. Reinitz (2009a) Canalization of Gene Expression in the *Drosophila* Blastoderm by Gap Gene Cross Regulation. *PLoS Biol* **7**: e1000049.

Manu, S. Surkova, A.V. Spirov, V.V. Gursky, H. Janssens, A.-R. Kim, O. Radulescu, C.E. Vanario-Alonso, D.H. Sharp, M. Samsonova and J. Reinitz (2009b) Canalization of Gene Expression and Domain Shifts in the *Drosophila* Blastoderm by Dynamical Attractors. *PLoS Comp Biol* **5**: e1000303.

Naldi A., D. Berenguier, A. Fauré, F. Lopez, D. Thieffry and C. Chaouiya (2009) Logical modelling of regulatory networks with GINsim 2.3. *BioSystems* **97**: 134-139.

Strogatz S.H. (2001) Nonlinear Dynamics and Chaos. Perseus Books, Cambridge, MA, USA.

Surkova S., D. Kosman, K. Kozlov, S. Manu, E. Myasnikova, A.A. Samsonova, A. Spirov, C.E. Vanario-Alonso, M. Samsonova and J. Reinitz (2008) Characterization of the Drosophila segment determination morphome. *Dev. Biol.* **313**(2): 844-862.

Yakoby N., C.A. Bristow, D. Gong, X. Schafer, J. Lemborg, J.J. Zartman, M.S. Halfon, T. Schüpbach and S.V. Shvartsman (2008) A combinatorial code for pattern formation in Drosophila oogenesis. *Developmental Cell* **15**: 725-737.