# Epigenetic plasticity and polarity of the embryo

**UMR3664 – Nuclear Dynamics**

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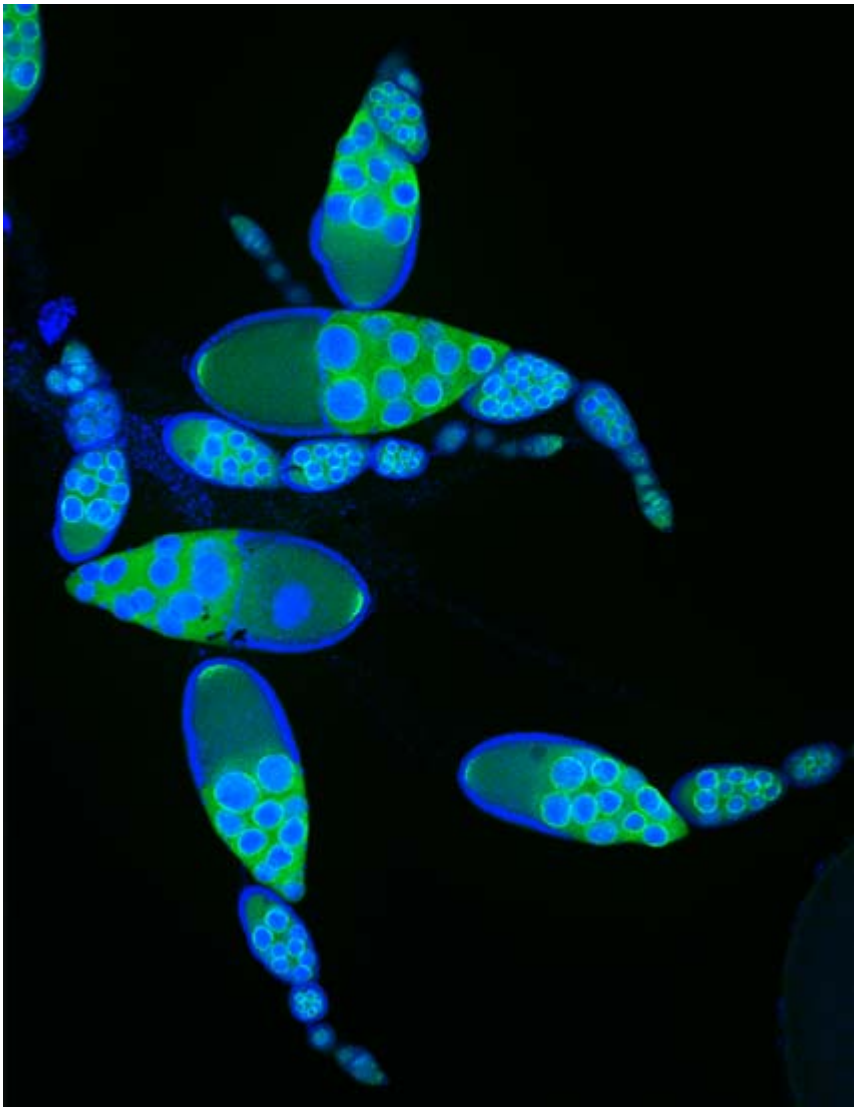
**Our work aims to elucidate how cells acquire and maintain their identity during development. This question is particularly critical in the context of cancer as, in tumors, cells have very frequently changed their identity. As in eukaryotes the "epigenome" predominantly determines cellular identity, we focus on the role of chromatin dynamics to maintain genome integrity and gene expression during development. Our model system, the *Drosophila* fruit fly, is ideally suited for classical and molecular genetic approaches, as it develops rapidly and is particularly well adapted for cutting-edge imaging. Our recent work focuses on the CAF-1 chromatin assembly factor and on the Bicoid transcription factor gradient.**

***The dynamics of chromatin during development*** In order to better understand the maintenance of chromatin structure across cell division, we focus on CAF-1, which allows assembly of H3/H4 dimers on newly replicated/repaired DNA and contributes to the formation/maintenance of heterochromatin. Our model system permits in vivo investigations on the whole organism during development. We investigate CAF-1 function in specific cell types with unique features that lack appropriate in vitro culture models. These cell populations include *i)* meiotic cells, which divide according to a unique process leading to haploid gametes and *ii)* stem cells, which divide asymmetrically and require a direct interaction with somatic cells of the niche for proper development.



*This image shows fruit fly ovarioles on which one can see a succession of ovarian chambers at different stages of development each containing one oocyte. The blue staining reveals DNA and the green staining the Aubergine protein. The image was produced by Marie Clémot. It was awarded in a scientific contest organized by the DEEP Labex and was published on 12th of August 2015 in "Le Monde".*

We characterized a novel evolutionary conserved HP1a interacting domain in the large subunit of Drosophila CAF-1. In contrast to complete loss of function, deletion of this domain does not perturb larval viability but reveals heterochromatin associated functions of CAF-1 in position effect variegation and homologous chromosome pairing during oocyte meiosis (Roelens et al., 2017). In addition, we demonstrated that female germline stem cells depleted of the CAF-1 large subunit still respond to niche signals but also express differentiation markers. In addition to this "identity crisis", these cells exhibit elevated levels of replicative stress and de-repression of

transposable elements. These DNA damage activate p53- and chk-2-dependent checkpoints leading to cell death and sterility. This work, performed in collaboration with the team of J.R. Huynh (Institut Curie, DEEP Labex), demonstrates that CAF-1-mediated chromatin dynamics plays a specific role to regulate stem cells identity and maintain their genome integrity (Clémot et al., 2018).

***The Bicoid morphogenetic gradient*** To better understand how cells acquire and maintain their identity during development, we concurrently focus on the transcriptional response downstream of the Bicoid morphogen gradient, a well characterized and simple paradigm patterning the embryonic antero-posterior axis. We combine integrative approaches from molecular genetics to quantitative imaging. In collaboration with physicists, we developed multi-scale imaging approaches, on fixed and living embryos, to understand the transcription process in a quantitative manner. To understand how the Bicoid system rapidly provides a precise transcriptional response despite the challenge imposed by very frequent mitoses, we adapted to the fly embryo the MS2 approach that allows RNA labelling in living cells and provides access to the transcription process at a single locus resolution in real time (Lucas et al., 2013). This pioneering work created a path to the temporal (4th) dimension to understand how reproducible transcription patterns can robustly emerge from a smooth gradient given the inherent stochastic nature of transcription. We successfully built an MS2 reporter reproducing endogenous expression of *hunchback*, the main Bcd target, at the onset of zygotic transcription (see the movie).

Using this tool, we discovered that *hunchback* transcription *per se* is stochastic in early fruit fly embryos. However, advanced data analysis indicated that this stochasticity cannot be explained by random firing of the polymerase (Poisson model) only, pointing towards more complex activation models (Desponds et al., 2016). We found that the shape of the boundary separating *hunchback* expressing from non-expressing nuclei is extremely sharp and consistent with the snapshots obtained from RNA FISH. Thus despite high nucleus-to-nucleus variability in transcription kinetics, the *hunchback* promoter is able to establish a sharp expression boundary that separates the anterior from the posterior of the embryo. Surprisingly, despite the absence of transcription during the frequent mitoses at this stage of development, it only takes 3 min at each interphase for the system to measure subtle differences in Bicoid concentration and produce a complete sharp border (Lucas et al., 2018). This rapid responsiveness is fascinating because it is almost ten times faster than predicted by theoretical models which have to integrate how distinct read-outs result from the trade-offs between the short time to establish a boundary, the observed steepness of this boundary and the positional resolution of nuclei on both sides of the boundary (Tran et al., 2018). This work results from an interdisciplinary collaboration with biophysicists (M. Coppey at the Institut Curie/UMR168 & C. Fradin at McMaster University, Hamilton, Canada) and theoreticians (A. Walczak at the ENS, Paris).

# Key publications

### Year of publication 2019

Ariane Ramaekers, Annelies Claeys, Martin Kapun, Emmanuèle Mouchel-Vielh, Delphine Potier, Simon Weinberger, Nicola Grillenzoni, Delphine Dardalhon-Cuménal, Jiekun Yan, Reinhard Wolf, Thomas Flatt, Erich Buchner, Bassem A Hassan (2019 Aug 27)
**Altering the Temporal Regulation of One Transcription Factor Drives Evolutionary Trade-Offs between Head Sensory Organs.**
*Developmental cell* : 780-792.e7 : DOI : S1534-5807(19)30658-6

### Year of publication 2018

Tanguy Lucas, Huy Tran, Carmina Angelica Perez Romero, Aurélien Guillou, Cécile Fradin, Mathieu Coppey, Aleksandra M Walczak, Nathalie Dostatni (2018 Oct 27)
**3 minutes to precisely measure morphogen concentration.**
*PLoS genetics* : e1007676 : DOI : 10.1371/journal.pgen.1007676

Huy Tran, Jonathan Desponds, Carmina Angelica Perez Romero, Mathieu Coppey, Cecile Fradin, Nathalie Dostatni, Aleksandra M Walczak (2018 Oct 12)
**Precision in a rush: Trade-offs between reproducibility and steepness of the hunchback expression pattern.**
*PLoS computational biology* : e1006513 : DOI : 10.1371/journal.pcbi.1006513

Marie Clémot, Anahi Molla-Herman, Juliette Mathieu, Jean-René Huynh, Nathalie Dostatni (2018 Aug 11)
**The replicative histone chaperone CAF1 is essential for the maintenance of identity and genome integrity in adult stem cells.**
*Development (Cambridge, England)* : DOI : dev161190

### Year of publication 2013

Tanguy Lucas, Teresa Ferraro, Baptiste Roelens, Jose De Las Heras Chanes, Aleksandra M Walczak, Mathieu Coppey, Nathalie Dostatni (2013 Jul 26)
**Live imaging of bicoid-dependent transcription in Drosophila embryos.**
*Current biology : CB* : 2135-9 : DOI : 10.1016/j.cub.2013.08.053

### Year of publication 2010

Aude Porcher, Asmahan Abu-Arish, Sébastien Huart, Baptiste Roelens, Cécile Fradin, Nathalie Dostatni (2010 Jul 29)

**The time to measure positional information: maternal hunchback is required for the synchrony of the Bicoid transcriptional response at the onset of zygotic transcription.**
*Development (Cambridge, England)* : 2795-804 : DOI : 10.1242/dev.051300