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Remodelling chromatin in worms

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Regulating gene expression is a question of sculpture. To get to the sequence information, proteins that activate and repress gene expression must reshape the DNA by removing or pushing aside some of its packaging material. Several large protein complexes can do this in the test tube. New experiments are now demonstrating how important these molecular sculptors are for development.

The packaging of a cell's DNA into chromatin is necessary to fit the vast length of the genome into the confined space of the nucleus. The packaging materials are spherical protein complexes that spool the DNA around themselves. These protein–DNA complexes are called nucleosomes and stack closely together to further compact the genetic material. But these packaging proteins pose a barrier to the many factors that need to gain access to the DNA template during fundamental processes like transcription, replication, recombination and repair. To alleviate this problem, the cell uses enzymes that hydrolyse ATP to keep the chromatin structure in a dynamic state that allows all the essential DNA-related processes to take place.

These ATP-dependent chromatin-remodelling enzymes have been studied in the biochemists'

test tubes for a number of years now and we have learned a great deal about how they move nucleosomes off, on and along the DNA. We know very little, however, about the function of ATP-dependent chromatin remodelling in the cell, let alone in a whole organism. Thomas von Zelewsky, Francesca Palladino and colleagues working in Fritz Müller's laboratory at the Institute of Zoology of the University of Fribourg, Switzerland, have now begun to close this gap by studying the role of two chromatin remodelling ATPases, CHD-3 and CHD-4, during development of the free-living roundworm *Caenorhabditis elegans*[1]. They show that ATP-dependent chromatin remodellers are not simply housekeeping enzymes but that they can play important roles in specific developmental pathways.

ATP-dependent chromatin remodelling

The cell uses two mechanisms to modulate chromatin structure. First, there are enzymes that post-translationally modify histones – the protein components of the nucleosomes – by acetylation, phosphorylation or methylation. These histone modifications either directly (by changing histone–DNA interactions) or indirectly (by attracting other chromatin remodellers) affect the structure of chromatin[2]. The second mechanism involves active, energy-dependent remodelling of chromatin[3,4].

Multisubunit complexes that use the energy derived from ATP hydrolysis to remodel chromatin *in vitro* have been isolated from a variety of species[4]. The first was the budding yeast (*Saccharomyces cerevisiae*) SWI/SNF complex, which harbours an ATPase called SWI2/SNF2. Homologues of this ATPase exist in higher eukaryotes[5], and chromatin-

remodelling complexes similar to the yeast SWI/SNF complex have been identified in flies and man. Yeast also has a second chromatin-remodelling complex, called RSC, containing Sth1, an ATPase related to SWI2/SNF2. To date, no Sth1-containing complexes have been found in higher eukaryotes.

A third chromatin remodelling ATPase, ISWI, was first identified in several complexes isolated from embryos of the fruit fly *Drosophila* (the complexes are called NURF, ACF and CHRAC). ISWI-containing complexes also exist in yeast, *Xenopus* and man.

The latest addition to the growing clan of ATP-dependent chromatin remodellers is a family of complexes containing a CHD ATPase. Members of this family were originally defined by their possession of a chromodomain and a DNA-binding domain in addition to an ATPase domain[6]. Three CHD ATPases are part of chromatin remodelling complexes: CHD1, CHD3 (also known as Mi-2a) and CHD4 (Mi-2b)[7–11].

Complexes containing CHD3 and CHD4 have been found in *Xenopus, Drosophila* and humans. They differ from other ATP-dependent chromatin remodellers in having a second chromatin-directed enzymatic activity, a histone deacetylase. So, CHD3/4-containing complexes combine the two strategies of chromatin remodelling – active, ATP-dependent remodelling and remodelling by post-translational modification of histones.

These four different families of ATP-dependent chromatin remodellers (SWI2/SNF2, Sth1, ISWI and CHD complexes) share some of their biochemical properties and differ in others, arguing that they might play redundant as well as specialised functions *in vivo*[3,4]. Several of the ATPase subunits alone have basic

chromatin remodelling activity when tested as recombinant proteins in the absence of other subunits[12–14]. These findings suggest that the other factors in chromatin remodelling complexes regulate the activity and quality of the chromatin remodelling reactions.

So, we are beginning to learn in considerable biochemical detail about how ATP-dependent chromatin remodellers go about their jobs, but how is this relevant to their functions in animals?

CHDs in flies

Johannes Kehle and Dirk Beuchle undertook the first detailed genetic analysis of a CHD chromatin remodelling ATPase in higher eukaryotes in Jürg Müller's laboratory, at the Max-Planck-Institut für Entwicklungsbiologie in Tübingen, Germany[15]. They found that *Drosophila* carrying homozygous mutations in their *dMi-2* gene (the *Drosophila* homologue of *CHD3* and *CHD4*) die during the larval stage of development. But these larvae do not display any obvious structural defects, probably because enough maternal dMi-2 mRNA is deposited in the eggs to carry them through the early larval stages.

Genetic interactions between *dMi-2* and the *hunchback* gene (whose protein product physically interacts with the dMi-2 protein) and between *dMi-2* and the *polycomb*-group gene *posterior sex combs* demonstrated that *dMi-2* participates in both *hunchback*- and *polycomb*-group-mediated gene repression *in vivo.* Both *hunchback* and the *polycomb*-group genes repress transcription of dMi-2, but they do so at different times: *hunchback* acts early in development to help establish repression of its target genes. Later, the *polycomb*-group gene

products help to maintain the repressed state in the appropriate tissues throughout development and adult life.

So, the findings of Kehle and colleagues suggested that CHD chromatin remodelling complexes might play a specific role in development, both in establishment and maintenance of the repressed state.

Thomas von Zelewsky and Francesca Palladino in Fritz Müller's lab in Switzerland have now studied the role of the two *C. elegans* CHD family members in the development of the worm[1]. Their results reinforce the idea that chromatin remodellers play distinct roles during development and organogenesis.

chd-4 is an essential gene

von Zelewsky and colleagues began by analysing what effect removal of the *chd-4* gene's function has in *C. elegans.* They first noted that, as in *Drosophila,* some maternal *chd-4* mRNA carries over into the embryo. Indeed, this maternal contribution is sufficient to allow a mutant embryo lacking a functional *chd-4* gene of its own to develop into adulthood. Not all is well with these animals, however, as almost all of them (98%) are sterile. Without the maternal contribution of *chd-4* mRNA, chd4 mutant embryos fail to complete their developmental programme and they arrest at an early larval stage.

Unlike *chd-4,* no maternal *chd-3* mRNA remains in the embryo, yet embryos lacking a functional *chd-3* gene develop normally with no obvious phenotype. A role for *chd-3* becomes apparent only in mutant embryos that express neither *chd-3* nor *chd-4;* in this special case, the maternal dose of *chd-4* mRNA is not sufficient

to get the embryo all the way through development. The embryos arrest during late larval stages. Thus, the Swiss team has demonstrated genetically that *chd-4,* but not *chd-3,* is an essential gene for the development of *C. elegans.* Nevertheless, *chd-3* must do something during development because the maternal dose of *chd-4* is not sufficient to see the double mutant through to adulthood.

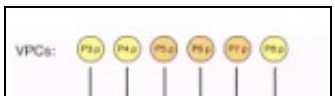
Despite the high amino acid sequence similarity between their protein products, *chd-3* cannot compensate for the complete loss of *chd-4* function. Nevertheless, *chd-3* and *chd-4* share some redundant functions during development (see below).

von Zelewsky and colleagues next looked at the expression patterns of *chd-3* and *chd-4* throughout development. This analysis was carried out by looking at the expression of a green fluorescent protein (GFP) gene under the control of the endogenous *chd-3* or *chd-4* promoters. GFP expression was detected in most cells of embryos, larvae and adults as might be expected for enzymes that play a role in chromatin remodelling, a process that is surely relevant for all cells.

Their observations that lack of *chd-4* expression in the zygote results in sterile animals and the occasional inappropriate induction of one of the vulva precursor cells (in 7% of cases) prompted them to investigate the possibility that *chd-3* and/or *chd-4* might be involved in vulval development.

ATP-dependent chromatin remodellers and vulval development

The vulva of *C. elegans* is formed from the



descendants of three (P5.p, P6.p, P7.p) of the six vulval precursor cells (called P3.p–P8.p) (Figure 1)[16,17]. In the wild-type animal P6.p, adopts the primary vulval cell fate, which is to generate eight descendants that form vulval tissue. P5.p and P7.p each adopts the secondary vulval cell fate, which is to generate seven descendants that form vulval tissue. The remaining three precursor cells adopt the tertiary fate and do not participate in vulva formation.

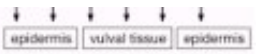


Figure 1
Three of the six vulval precursor cells (VPCs; P5-7.p; orange) develop into vulval tissue in response to an inductive signal that is transduced by the RTK–Ras pathway. The RTK–Ras pathway in the remaining VPCs (yellow) is antagonised by two redundant classes of genes, *synMuvA* and *synMuvB*. Combined mutations of *synMuvA* and *synMuvB* class genes results in the P3-4.p and/or the P8.p cells inappropriately adopting vulval fates resulting in the multivulva phenotype.

The *synthetic multivulva* (*synMuv*) genes negatively regulate vulval induction[18,19]. The *synMuv* genes fall into two classes, A and B. Loss-of-function mutations in both a class A gene and a class B gene result in a multivulva phenotype. The formation of multiple vulvas in these mutants is the consequence of the precursor cells P3.p, P4.p or P8.p adopting vulval fates. The fact that two mutations are required to produce this phenotype has been interpreted as the consequence of two independent and redundant pathways that antagonise vulval cell fates[18].

The Swiss team generated animals mutant for *chd-4* and a *synMuv* gene of either the A or the B class and checked for the appearance of the multivulva phenotype.

Combination of a *chd-4* mutation and a class B mutation did not produce a significant effect. By contrast, combination of a *chd-4* mutation with a class A mutation generated the multivulva phenotype with high penetrance (in 73–82% of the animals). These results identify *chd-4* as a

member of the *synMuvB* class of genes.

Interestingly, two putative subunits of the *C. elegans* CHD3/4 complexes, including the homologue of histone deacetylase 1 (HDAC1), are also components of the synMuvB pathway[20]. Florence Solari and Julie Ahringer from the Wellcome/CRC Institute in Cambridge (England, UK) had previously identified *chd-3* and *chd-4* as *synMuv* genes by using RNA interference (RNAi)[21,22]. Their results suggested that *chd-3* and *chd-4* act in both *synMuv* groups A and B. The genetic data of the Müller lab, however, now place *chd-4* in the synMuvB pathway.

The fact that the *chd-3* allele tested by the Müller lab does not show a synMuvB phenotype suggests either that it retains a partial function or that the RNAi phenotype seen by the Ahringer lab is a result of both the *chd-3* and *chd-4* genes being inactivated due to their close sequence similarity (for a discussion of RNAi in *C. elegans see* the mini review by Michel Labouesse in Issue 4 (March 2001) of *The ELSO Gazette*).

In *C. elegans,* development of the vulva requires signal transduction through a receptor tyrosine kinase (RTK)–Ras–MAP kinase pathway[23]. von Zelewsky and colleagues tested what happens when this pathway, which is required for induction of the vulval precursor cells, is inactivated; animals carrying a mutant version of the receptor tyrosine kinase gene *let-23,* the *chd-4* mutation and a mutation in a *synMuvA* gene no longer displayed the multivulva phenotype. This confirmed that the synMuv phenotype requires a functional signal transduction pathway.

The CHD4 complex at work

So, both the Müller and Ahringer labs have shown that *chd-4* is involved in vulva development, but how does the gene exert its effect?

The available biochemical data suggests that CHD-4 is part of a multisubunit chromatin-remodelling complex involved in repressing transcription. A number of DNA-binding transcriptional repressors interact with components of this complex[9,15,24]. von Zelewsky and colleagues suggest that the *C. elegans* CHD-4 complex represses genes that specify vulval cell fate. (Figure 2)

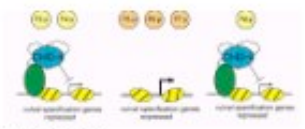


Figure 2
A model for CHD-4 function in vulva development. In three of the VPCs (yellow) a CHD-4 chromatin remodelling complex is recruited to vulval specification genes that are then repressed preventing adoption of the vulval fate. In the three induced VPCs (orange) the vulval specification genes are expressed either due to an inactive CHD-4 complex or due to failure to recruit the complex to the genes.

The DNA-binding repressor that could recruit this complex is not known. But given the fact that the transcriptional repressor LIN-35 (the *C. elegans* homologue of Retinoblastoma) is a *synMuvB* gene[20], it is tempting to speculate that the CHD-4 complex functions, at least in part, through LIN-35-regulated target genes. At present, there is no biochemical data, however, to confirm a physical interaction between CHD3/4 and Retinoblastoma in any species.

Although the synMuv pathway opposes an RTK–Ras pathway it is not known how these pathways interact. It is conceivable that CHD-4 represses genes that encode components or targets of the RTK–Ras pathway. It is also possible that the RTK–Ras pathway inactivates a CHD-4 complex or a DNA-bound factor that recruits CHD-4 through phosphorylation.

Clearly, much more work needs to be done to understand fully how chromatin-remodelling

complexes are integrated into signalling and developmental pathways.

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