Chromatin-modifying complexes are important for transcriptional control, but their roles in the regulation of development are poorly understood. Here, we show that five Caenorhabditis elegans members of the NURD chromatin remodelling complex inhibit vulval development through both the synMuvA and synMuvB pathways (hda-1, rba-1, lin-53, chd-3 and chd-4); a further two members, the MTA1-related genes egr-1 and egl-27, act only in the synMuvA pathway. We propose that the synMuvA and synMuvB pathways function redundantly to recruit or activate a core NURD complex, which then represses vulval developmental target genes by local histone deacetylation. These results emphasise the importance of chromatin regulation in developmental decisions. Furthermore, inhibition of Ras signaling suggests a possible link between NURD function and cancer.

In a mutant in which the Ras pathway is overactive because of a gain-of-function mutation in the let-60 Ras gene, excess vulval tissue is made [12]. To further explore whether egr-1 functions to antagonise Ras signaling, we asked whether loss of egr-1 activity would enhance the Muv phenotype of let-60(n1046gf). Indeed, egr-1(RNAi);let-60(n1046gf) animals are significantly more Muv than let-60(n1046gf) alone (92%, n = 199 versus 64%, n = 283). Injection of dsRNA of an unrelated gene (vab-7) had no effect on the Muv frequency (data not shown). These results indicate that egr-1 has a role in repressing the Ras pathway.

We found that two alleles that were previously attributed to the complex locus lin-40, called s1593 and s1669, introduce stop codons into the egr-1 coding sequence (see Supplementary material). Analyses of lin-40 alleles showed that they identify at least three different genes (F.S., B. Johnsen, D. Baillie and J.A., unpublished observations); s1593 and s1669 will now be called alleles of egr-1. Both egr-1(s1593) and egr-1(s1669) become sterile adults with abnormal gonads, similar to egr-1(RNAi).
are egr-1 and egl-27 mutants (Table 1). This shows that double egr-1(RNAi) percentage of Muv animals over triple mutants show a large increase in the percentage of Muv animals over synMuvB egl-27(RNAi) egr-1(RNAi) genes (Table 1). However, a strong interaction with synMuvA and synMuvB does not have a strong interaction with egl-27 egr-1(s1593) egr-1(s1593) lin-37;lin-15B(RNAi);egr-1(s1593) lin-15B(RNAi) mutants, but not egr-1(s1593) double mutants are Muv at high frequency (39%, n = 109). To confirm that chd-3 egr-4* EGR-1 is a ubiquitous nuclear protein (see Supplementary material).

Table 1

<table>
<thead>
<tr>
<th>Gene used for RNAi</th>
<th>Method used</th>
<th>Wid type</th>
<th>synMuvA</th>
<th>synMuvB</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>0% (400)</td>
<td>0% (170)</td>
<td>0% (180)</td>
</tr>
<tr>
<td>egr-1</td>
<td>Inj</td>
<td>3% (0–6%, 254)</td>
<td>2% (0–6%, 176)</td>
<td>3% (0–6%, 140)</td>
</tr>
<tr>
<td>egr-1</td>
<td>Soak</td>
<td>0% (0–0%, 158)</td>
<td>1% (0–1%, 162)</td>
<td>–</td>
</tr>
<tr>
<td>egl-27</td>
<td>Soak</td>
<td>&lt;1% (0–0%, 371)</td>
<td>0% (0–0%, 169)</td>
<td>–</td>
</tr>
<tr>
<td>egr-1 + egl-27</td>
<td>Soak</td>
<td>0% (0–0%, 184)</td>
<td>0% (0–0%, 98)</td>
<td>–</td>
</tr>
<tr>
<td>lin-53</td>
<td>Soak</td>
<td>0% (0–0%, 136)</td>
<td>25% (22–27%, 112)</td>
<td>–</td>
</tr>
<tr>
<td>rba-1*</td>
<td>Soak</td>
<td>0% (0–0%, 145)</td>
<td>2% (0–2%, 129)</td>
<td>1% (76)</td>
</tr>
<tr>
<td>hda-1*</td>
<td>Soak</td>
<td>0% (0–0%, 169)</td>
<td>21% (18–22%, 100)</td>
<td>–</td>
</tr>
<tr>
<td>chd-3</td>
<td>Soak</td>
<td>&lt;1N (0–1%, 177)</td>
<td>30% (22–47%, 89)</td>
<td>3% (37)</td>
</tr>
<tr>
<td>chd-4</td>
<td>Soak</td>
<td>0% (0–0%, 207)</td>
<td>23% (15–32%, 129)</td>
<td>1% (60)</td>
</tr>
<tr>
<td>chd-3 + chd-4</td>
<td>Soak</td>
<td>0% (0–0%, 137)</td>
<td>20% (41)</td>
<td>1% (74)</td>
</tr>
</tbody>
</table>

Figures in bold are percentage Muv, with the range of results from multiple RNAi experiments as appropriate (up to 6 replicates) and the total number of animals scored in parentheses. Inj. RNAi was performed by injection; soak. RNAi was performed by soaking. Experiments were carried out at 22°C. Wild-type, synMuvA (in-15A), and synMuvB (in-26 and in-9) genotypes used in the RNAi experiment. Dash, not done. The following animals (Figure 1f,g and data not shown). Expression of an egr-1::gfp (green fluorescent protein) reporter gene suggests that EGR-1 is a ubiquitous nuclear protein (see Supplementary material).

To confirm that egr-1 acts in the synMuvA pathway, we constructed double mutants between egr-1(s1593) and either a synMuvA mutant (lin-15A(n767)) or a synMuvB mutant (lin-53). The egr-1(s1593) animals are Muv at low frequency (5%, n = 100), similar to egr-1(RNAi) (Table 1 and Figure 1f,g). We found that egr-1(s1593);lin-37 double mutants, but not egr-1(s1593);lin-15A double mutants, are Muv at high frequency (39%, n = 108 versus 1%, n = 119; Figure 1h). Furthermore, egr-1(s1593);lin-15B(RNAi) are often Muv (26%, n = 53; lin-15B is a synMuvB gene) whereas egr-1(s1593);lin-15A(RNAi) are not (0%, n = 43). Therefore, the RNAi and mutant data both indicate that egr-1 is a synMuvA gene.

Because egr-1 and egl-27 are functionally redundant in embryonic patterning [8], we investigated whether egl-27 also has a role in vulval development. On its own, egl-27 does not have a strong interaction with synMuvA or synMuvB genes (Table 1). However, egr-1(RNAi);egl-27(RNAi);synMuvA/B double mutants show a large increase in the percentage of Muv animals (Table 1). This shows that egl-27 and egr-1 are partially redundant in vulval development, although egr-1 appears to be more important.

EGR-1 and EGL-27 are similar to one component of the NURD complex, Mta1. We next explored other C. elegans members of the NURD complex for roles in vulval development. Besides MTA1, the NURD complex contains HDAC3 and HDAC2 (histone deacetylases), RbAp46/48 (RB-associated proteins), CHD3 and/or CHD4 (highly similar chromodomain helicase proteins), MDR3 (similar to methyl-CpG-binding proteins) and several uncharacterised polypeptides [1–5,13]. In C. elegans, two genes encoding RbAp46/48 homologues, rba-1 and hda-2 (previously known as rba-2), and three histone deacetylases, hda-1, hda-2 and hda-3, have been previously described [11,14]. In a search of the current C. elegans genome sequence (over 99% complete), we identified two good matches to CHD3 and CHD4 (T14G8.1 and F26F12.7), each is 48% identical to both human CHD3 and CHD4, and we have named the corresponding genes chd-3 and chd-4, respectively. At present, there is no clear C. elegans match to MDR3.

Previous work showed that hda-1 and the RbAp46/48 gene lin-53 are synMuvB genes [11]. Both are predicted to encode proteins in the NURD complex. However, we found that egr-1, encoding one member of this complex, is a
synMuvA gene. To address this conundrum, we performed RNAi with hda-1 and lin-53 in wild-type, synMuvA and synMuvB backgrounds. Injection of dsRNA corresponding to either gene causes embryonic lethality ([8] and data not shown). To overcome this technical difficulty, we performed the RNAi experiments by soaking first-stage larvae in dsRNA; this has been shown to be an effective alternative method to injection of mothers with dsRNA, although it produces a slightly weaker effect [15].

Surprisingly, hda-1(RNAi) and lin-53(RNAi) have both synMuvA and synMuvB properties (Table 1). For example, as previously reported [11], lin-53 behaves as a synMuvB gene, because lin-53(RNAi) produces Muv animals in a synMuvA background but not in wild-type animals (Table 1). However, lin-53(RNAi); synMuvB animals are also Muv (Table 1), indicating that lin-53 is also a synMuvA gene. The other RbAb46/48 homologue, rba-1, also has both synMuvA and synMuvB properties (Table 1). The genes do not appear to be redundant, as the frequency of Muv is only additive (and not greater as would be expected for redundant genes) when rba-1 and lin-53 are inactivated together. These results suggest that each of the two C. elegans RbAb46/48 homologues functions in both synMuvA and synMuvB pathways.

The hda-1 gene was previously shown to have synMuvB characteristics, but was not tested for synMuvA activity [11]. We found that hda-1(RNAi); synMuvA animals are Muv (Table 1), confirming that hda-1 is a synMuvB gene. In a synMuvB background, hda-1(RNAi) also produces Muv animals at high frequency (Table 1). Therefore, like the RbAp48 homologues, hda-1 appears to act in both pathways. Neither hda-2(RNAi) nor hda-3(RNAi), nor RNAi to both together, produced Muv animals in any background (data not shown and [14]).

Finally, we tested whether chd-3 and chd-4 act in either synMuv pathway: chd-3(RNAi) and chd-4(RNAi) each produces a significant percentage of Muv animals in both synMuvA and synMuvB mutant backgrounds (Table 1), indicating that chd-3 and chd-4 act in both pathways. The chd-3 and chd-4 genes appear to have non-redundant functions, because removing both together does not increase the frequency of Muv animals (Table 1).

In summary, we have shown that members of the NURD chromatin-remodelling complex inhibit vulval development within both the synMuvA and synMuvB pathways. Biochemical analyses in a number of systems have identified a NURD complex with the composition described above [1-5,13]. The simplest interpretation of our finding is that of the involvement of each NURD member in repression of vulval development is that these proteins act together in a single complex, but it is possible that individual members might have independent functions in the vulva as well.

Complexes such as NURD are proposed to interact with sequence-specific transcription factors, resulting in chromatin changes and repression of target genes [1,3–5]. In C. elegans, EGR-1 and EGL-27 appear to act in the synMuvA pathway (Figure 2). Interaction of the core NURD complex with either the synMuvA or synMuvB pathway would be sufficient for vulva-specific activity. Unexpectedly, the two MTA1 homologues, EGR-1 and EGL-27, which were thought to be integral to the NURD complex, appear to act only in the synMuvA pathway. This raises the possibility that EGR-1 and EGL-27 may function within the synMuvA pathway to recruit a partial NURD complex. Likewise, the Rb-like gene lin-35 functions only in the synMuvB pathway [11]. Rb is a transcriptional co-repressor, and recent biochemical studies have linked Rb to the histone deacetylase HDAC1 and a CHD3/4-like protein Mi2β [16–19]. Therefore, LIN-35 could be a synMuvB adapter. Possilbe transcription factor targets of the complex are LIN-1 and LIN-33, which are vulval development repressors regulated by Ras signaling [20].

Previous screens for vulval development genes failed to identify most of the genes we describe here. A likely


