Ssrp1a controls organogenesis by promoting cell cycle progression and RNA synthesis

Katarzyna Koltowska1,*, Holger Apitz2, Despina Stamataki1, Elizabeth M. A. Hirst3, Heather Verkade4, Iris Salecker5 and Elke A. Ober1,6

SUMMARY
Tightly controlled DNA replication and RNA transcription are essential for differentiation and tissue growth in multicellular organisms. Histone chaperones, including the FACT (facilitates chromatin transcription) complex, are central for these processes and act by mediating DNA access through nucleosome reorganisation. However, their roles in vertebrate organogenesis are poorly understood. Here, we report the identification of zebrafish mutants for the gene encoding Structure specific recognition protein 1a (Ssrp1a), which, together with Spt16, forms the FACT heterodimer. Focussing on the liver and eye, we show that zygotic Ssrp1a is essential for proliferation and differentiation during organogenesis. Specifically, gene expression indicative of progressive organ differentiation, together with its dynamic spatiotemporal expression, ensure organ-specific differentiation and proportional growth, which are crucial for the forming embryo.

KEY WORDS: Ssrp1, Cell cycle, Hdac, Liver, Organogenesis, Zebrafish, Drosophila

INTRODUCTION
Expansion of progenitor pools and their tissue-specific differentiation are central for the formation of functional organs. Complex signalling cascades controlling organ growth and differentiation have been identified, but the associated basic cellular processes, such as regulation of DNA accessibility, in the developing embryo are still poorly understood. DNA accessibility, allowing the passage of RNA polymerase II and replication forks, is pivotal for coordinated gene expression and cell proliferation. Histone chaperones mediate DNA accessibility by structural destabilisation of nucleosomes and deconvolution of higher-order chromatin structure (Ransom et al., 2010). One histone chaperone, the FACT (facilitates chromatin transcription) complex consists of Spt16 (suppressor of Ty16; also known as Supt16h in zebrafish) and Ssrp1 (Structure specific recognition protein 1), which are both essential for nucleosome reorganisation and conserved across metazoans and plants (Formosa, 2008; Winkler and Luguer, 2011). Metazoan Ssrp1 contains a C-terminal high mobility group box (HMGB), whereas its yeast homologue Pob3 solely encodes the N-terminal domains and the N-terminal high mobility group box (HMGB), whereas its yeast homologue Pob3 is required for cell viability, whereas Nhp6 is not essential (Malone et al., 1991; Wittmeyer et al., 1999; Brewster et al., 2005) (for genotyping details, see Zebrafish International Resource Center).

Tightly controlled DNA replication and RNA transcription are essential for differentiation and tissue growth in multicellular organisms. Histone chaperones, including the FACT (facilitates chromatin transcription) complex, are central for these processes and act by mediating DNA access through nucleosome reorganisation. However, their roles in vertebrate organogenesis are poorly understood. Here, we report the identification of zebrafish mutants for the gene encoding Structure specific recognition protein 1a (Ssrp1a), which, together with Spt16, forms the FACT heterodimer. Focussing on the liver and eye, we show that zygotic Ssrp1a is essential for proliferation and differentiation during organogenesis. Specifically, gene expression indicative of progressive organ differentiation, together with its dynamic spatiotemporal expression, ensure organ-specific differentiation and proportional growth, which are crucial for the forming embryo.

Here, we report that the FACT component Ssrp1a is essential for tissue differentiation and growth in vertebrate organogenesis. In the developing zebrafish liver and eye, zygotic loss of Ssrp1a results in impaired RNA transcription, defective S phase progression and cell death. Using cross-species rescue experiments, we examine the importance of the HMGB domain of Ssrp1a, as well as paralogous Ssrp1b, in metazoan organogenesis.

MATERIALS AND METHODS
Fish stocks
The following strains were bred under standard conditions (Westerfield, 2000): Tg(XIIEef1a:GFP)854 (Field et al., 2003), clamped819, clampeds819, cecyil: Tg(Efa:mKO2-Ced1(1/190))m105b, Tg(Efa:maG-zGem(1/100))m106b (Sugiyama et al., 2009) and tp53s819 (Berghmans et al., 2005) (for genotyping details, see Zebrafish International Resource Center).

Positional cloning of clamped819
Meiotic mapping followed standard protocols (Geisler, 2002). Sibling and cmp819 mutant CDNAS were sequenced for ssrp1a, rad9a, zgc:113229, med19a and slc43a1a. For genotyping of homozygous cmp819 embryos, dCaps Finder 2.0 (http://helix.wustl.edu/dcaps/dcaps.html) was used to design primers creating a Hinfl restriction site including the ssrp1a819 mutation. The following primers were used for PCR amplification from genomic DNA: forward 5’-AGCTTATTAGACACAGATGG-3’ and reverse 5’-CAGTTYCCTAATTCACGCCCTTGAGC-3’. PCR amplification and subsequent Hinfl digest from homozygous mutant, wild-type and heterozygous embryos confirmed the identified lesion (supplementary material Fig. S1). ‘MO-ssrp1a’ (MO-ATG-ssrp1a, 5’-CGTTAAAATCCAGAGTGTCTCCCAT-3’; Gene Tools) and standard ‘MO-control’ were injected into one-cell-stage wild-type or Tg(XIIEef1a:GFP)854 zebrafish.
Zebrafish immunostaining and in situ hybridisation

Labelling was performed as described (Ober et al., 2006). The following antibodies were used: rabbit anti-Prox1 (1:1000; Chemicon), mouse anti-2F11 (1:1000; gift from J. Lewis, Cancer Research UK, London, UK), mouse anti-BrdU [1:20; Developmental Studies Hybridoma Bank (DSHB)] and rabbit anti-cleaved Caspase 3 (1:75; Cell Signaling Technology). Fluorescently conjugated secondary antibodies were obtained from Jackson Laboratories. Staining was visualised with a Zeiss LSM5 Pascal Exciter confocal microscope. Images were processed and cell numbers determined with Velocity image analysis software (Improvement).

The following probes were used for in situ hybridisation: aht5 (also known as aht07) (Masai et al., 2000), ceruloplasmin (Korzh et al., 2001), ceca (Shkumatava et al., 2004), foxa1 (Odenthal and Nüsslein-Volhard, 1998), group specific component (Noël et al., 2010), mdm2 (Chen et al., 2005), p21 [cyclin-dependent kinase inhibitor 1a (cdkia1a)] (Chen et al., 2005), pcn3 (Leung et al., 2005) and pftl (Lin et al., 2004).

BrdU incorporation assay

Embryos were incubated for 30 minutes at 28.5°C in 10 mM 5-bromo-deoxyuridine (BrdU) with 15% DMSO in embryo medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl2, 0.33 mM MgSO4) and prior to immunolabelling were treated with 2 M HCl for one hour at room temperature.

Global RNA labelling

Embryos were incubated in 10 mM 5-ethynyluridine (EU; Invitrogen) and analysed by fluorescence-activated cells sorting (FACS) for Global RNA labelling.

Mitotic index analysis

Foregut-containing tissue was isolated manually in Hank’s balanced salt solution with 5% fetal calf serum on ice and passed through a 40 μm filter. Single-cell suspensions were stained with Vybrant DyeCycle Ruby (Invitrogen) and analysed by fluorescence-activated cells sorting (FACS).

Drosophila molecular biology, genetics and histology

Zebrafish genes were cloned into a modified pKC26 UAS vector containing an attB site and a 3' V5 epitope-tag sequence. Plasmids were inserted into the attP site-containing locus VIE-260b to generate transgenic lines.

Clamped mutations disrupt an orthologue of Ssrfp1

Meiotic recombination mapping placed the cmp819 mutation on linkage group 14 in a 340-kb genomic region between markers mindin1 (spon2a) and rs41174000 (primers amplifying a ~160 bp product in an intron of cmp1: F: 5'-GCATAAAAACCTGC-3'; R: 5'-GCAGAGGAGGATGAGAAC-3') (Chen et al., 2005). Sequencing of five candidate genes within this region revealed a T-to-A base pair substitution at position 309 of cmps819 (100% = n=10, respectively; Fig. 1E-H). In addition, group specific component (ge), marking late hepatocyte differentiation (Noël et al., 2010), was not detected in cmp819 mutants and overall EU incorporation levels were lower (Fig. 1R-R'), indicating a general defect in RNA transcription. Consistently, actively transcribed genes with a short mRNA half-life, such as those encoding cyclins (Ohtani et al., 1995), represent some of the first genes exhibiting mRNA transcription defects (supplementary material Fig. S4L-M).

RESULTS AND DISCUSSION

The clamped819 mutation disrupts organ growth and differentiation

The clamped819 (cmp819) mutant was previously identified in a forward genetic screen for factors essential for endodermal organogenesis (Ober et al., 2006). cmp819 embryos display liver and pancreas hypoplasia at 48 hours post-fertilisation (hf, Fig. 1A-D), and smaller fins, head and eyes from 40 hf (Fig. 2B-E). Liver progenitors arise from the foregut endoderm and aggregate into the liver bud (Field et al., 2003). Concomitant with liver-bud outgrowth, hepatoblasts begin to differentiate into functional hepatocytes and biliary epithelial cells. From about 50 hf, the liver grows rapidly. Quantification of hepatic progenitors in cmp819 mutants using Prox1 expression showed a 40% reduction at 48 hf (Fig. 1B-D). ceruloplasmin (cp), which encodes a plasma protein in differentiating hepatoblasts (Korzhe et al., 2001), is expressed in cmp819 mutant livers at 48 hf, but is not maintained at 96 hf (100%, n=18 and n=10, respectively; Fig. 1E-H). In addition, group specific component (ge), marking late hepatocyte differentiation (Noël et al., 2010), was not detected in cmp819 mutants (100%, n=15; Fig. 1J-I). Hence, hepatic differentiation fails to progress in cmp819 mutants. Moreover, pan-endodermal expression of the transcription factor foxa1 in cmp819 mutants is similar to wild type at 40 hf (100%, n=12; Fig. 1K,L), but undetectable at 72 hf (100%, n=9; Fig. 1M,N). This is due to defects in transcription and not tissue loss, as Tg(XIIEef1a1:GFP)852 labels the digestive system in controls and cmp819 mutants at 72 hf (100%, n=10; Fig. 1O,P). In wild-type embryos, EU labelling of newly synthesised RNA (Jao and Salic, 2008) was detected in all nuclei with higher levels in transcriptionally active sites at 48 hf (Fig. 1Q-Q'). These foci were largely absent in cmp819 mutants and overall EU incorporation levels were lower (Fig. 1R-R'), indicating a general defect in RNA transcription. Consistently, actively transcribed genes with a short mRNA half-life, such as those encoding cyclins (Ohtani et al., 1995), represent some of the first genes exhibiting mRNA transcription defects (supplementary material Fig. S4L-M). Altogether, this suggests that cmp819 affects a key factor mediating gene transcription in the differentiating zebrafish liver.

qPCR

Quantitative PCR (qPCR) was performed using primer sets for ssrp1a and ssrp1b (ssrp1aIF 5'-CCTCATCCTTCTGTCCTAAA-3'; ssrp1aFR 5'-TCTTCACCTCACCTCTGTGTCCT-3'; ssrp1BF 5'-TCCGGCTCTTCTTTGATGAGT-3'; ssrp1BR 5'-CTCGAGTGATCCTGATGTAG-3'; rpl13F 5'-TCTCGGAGACTGTAAGGATGTC-3'; rpl13R 5'-AGACGCACAATCCTGAGAGAC-3'). mRNA was extracted from five to ten embryos and processed. qPCR (60°C annealing temperature) was carried out using ABsolute SYBR Green Mix (Thermo) on the ABI Prism 7000 RTPCR Detection system. Expression values were normalized using rpl13 and compared using Student’s t-test. Unless otherwise indicated, three independent experiments were assessed with three samples each.
Based on these validations, the cmp mutants are referred to as ssrp1as819 and ssrp1au428. All experiments were performed with ssrp1as819, as both alleles display comparable phenotypes.

ssrp1a is expressed ubiquitously in the early embryo and from ~36 hpf in proliferative tissues, such as the liver, eyes and fins (Fig. 2H-L). Quantitative RT-PCR (qPCR) analysis revealed high maternal ssrp1a mRNA levels (2.5 hpf), whereas zygotic expression is much lower (>3 hpf; Fig. 2M). Inhibiting translation of maternal ssrp1a mRNA by MO injection into Tg(XIEef1a1:GFP)s854 embryos does not produce stronger phenotypes compared with ssrp1as819 mutants (Fig. 2B-G), suggesting that maternal Ssrp1a protein compensates for Ssrp1a loss during early development. Alternatively, the zebrafish Ssrp1 paralogue Ssrp1b (81% identity)
might act redundantly (supplementary material Fig. S1B), as it is expressed throughout development, albeit at up to 98% lower levels than ssrp1a (Fig. 2M).

**Ssrp1a promotes cell cycle progression in the digestive system and eye**

Hepatic growth arrests in ssrp1a<sup>as19</sup> mutants at ~48 hpf. BrdU incorporation experiments revealed that at 36 hpf proliferation rates in ssrp1<sup>P18</sup> mutants (n=10) are similar to those in controls (n=13), but are reduced by ~10% at 38 hpf and by 87% at 49 hpf (n=10; Fig. 3A-C). This indicates that Ssrp1a promotes hepatic cell divisions. To determine the Ssrp1a-dependent cell cycle phase, mutant and sibling livers were analysed in the transgenic cecyl background labelling S/G<sub>2</sub>/M phase cells in green and G<sub>1</sub> phase cells in red (Sugiyama et al., 2009). At 38 hpf, 55-60% hepatoblasts are found in G<sub>1</sub> phase and ~40% in S/G<sub>2</sub>/M phase in controls and ssrp1<sup>as19</sup> mutants (Fig. 3D,E,H). This distribution changes significantly in ssrp1a<sup>as19</sup> mutants by 48 hpf, with 30% of cells in G<sub>1</sub> phase and 62% in S/G<sub>2</sub>/M phase (n=7; Fig. 3F-H), indicating that Ssrp1a-deficient hepatoblasts accumulate in S/G<sub>2</sub>/M phase. Next, we examined single cell suspensions from the foregut domain of Tg(XIEof1a:GFP)<sup>I854</sup> embryos using FACS. Measuring the DNA content at 42-44 hpf revealed 18.5% fewer Ssrp1a-deficient endodermal cells in G<sub>1</sub> phase and, conversely, 10.5% more in S phase compared with wild type (Fig. 3I). This indicates that Ssrp1a is required for S phase progression during development, consistent with in vitro results reporting Ssrp1 functions in DNA elongation (Abe et al., 2011).

Our data indicate that Ssrp1a-deficient cells accumulate in S phase, probably owing to more cells entering or stalling in S phase. A role for Ssrp1a during DNA synthesis, rather than at subsequent checkpoints prior to mitosis, is corroborated by fewer cells incorporating BrdU, and the intact, not polyploid appearance of nuclei in ssrp1a<sup>as19</sup> livers (Fig. 1Q,R). FACT interacts with the minichromosome maintenance (MCM) helicase in initiation and elongation of DNA synthesis (Gambaru et al., 2006; Tan et al., 2006) and, consistent with this, cell cycle defects in ssrp1a<sup>as19</sup> mutants resemble the ones in Mcm5-depleted zebrafish embryos (Ryu et al., 2005). Intriguingly, Spt16-depleted yeast exhibits a G<sub>1</sub> phase delay, pointing to specific functions of either FACT component (Morillo-Huesca et al., 2010). Similarly, human SSRP1 and SPT16 (SUPT16H) can regulate largely overlapping, but also distinct targets (Li et al., 2007).

To determine the specificity of different chromatin-remodelling factors on organ growth, we examined hdac<sup>P146</sup> mutants exhibiting similar liver size defects (Noël et al., 2008). Hdac1 mediates chromatin compaction and cell cycle progression from G<sub>1</sub> to S phase (Yamaguchi et al., 2010), raising the possibility that its loss might counterbalance factors on organ growth, we examined single cell suspensions from the foregut domain of Tg(XIEof1a:GFP)<sup>I854</sup> embryos using FACS. Measuring the DNA content at 42-44 hpf revealed 18.5% fewer Ssrp1a-deficient endodermal cells in G<sub>1</sub> phase and, conversely, 10.5% more in S phase compared with wild type (Fig. 3I). This indicates that Ssrp1a is required for S phase progression during development, consistent with in vitro results reporting Ssrp1 functions in DNA elongation (Abe et al., 2011). Compared with the liver, Ssrp1a is required earlier in retina differentiation and proliferation (~36 hpf; supplementary material Fig. S3), indicating similar, but temporally distinct, requirements for zygotic Ssrp1a in a tissue-specific manner. Liver and eye progenitors lacking Ssrp1a undergo apoptosis following S phase defects (supplementary material Fig. S3K-M and Fig. S4). Thus, in ssrp1a<sup>as19</sup> mutants, incomplete DNA synthesis may trigger a DNA-damage response. Indeed, increased Tp53-target gene expression (Vogelstein et al., 2000) (supplementary material Fig. S4H-K) and a partial rescue of cell death in ssrp1a;tp53 mutants (supplementary material Fig. S4F,G) showed that Tp53-dependent and -independent signalling is activated in Ssrp1a-deficient embryos. Human FACT activates TP53 following DNA damage (Keller et al., 2001) and in Ssrp1-deficient mice apoptosis is solely mediated by Tp53-dependent pathways (Cao et al., 2003). By contrast, our data in zebrafish suggest that cell death is not mediated exclusively via Tp53, but might include alternative pathways, highlighting the complexity of the molecular mechanisms following replication stress in different vertebrates.

**Fig. 3. Ssrp1a promotes cell cycle progression.** (A-C) BrdU incorporation is reduced in ssrp1a<sup>as19</sup> mutant livers from 38 hpf onwards (outlined). Error bars represent s.d. (D-G) Transgenic cecyl expression marks G<sub>1</sub> phase cells in red and S/G<sub>2</sub>/M phase cells in green. (H) Quantification of hepatoblast proliferation shows a reversed distribution in controls and ssrp1a<sup>as19</sup> mutants at 48 hpf. (I) FACS analysis of 42-44 hpf foregut endoderm shows an increase of cells in S phase and a decrease of those in G<sub>1</sub> phase in ssrp1a<sup>as19</sup> mutants. A-B,D-G are confocal projections of ventral views; all anterior to the top. *P<0.05, **P<0.005, determined by unpaired Student’s t-test.
Full-length ssrp1a and ssrp1b, as well as ssrp1a<sup>1-518</sup>, can compensate for the loss of Drosophila Ssrp

To elucidate the role of the HMGB domain, we performed cross-species rescue experiments using the Drosophila eye. The fly genome has one Ssrp gene (Shimozima et al., 2003), which encodes an HMGB-containing protein, similar to zebrafish Ssrp1a and Ssrp1b (supplementary material Fig. S1). Knockdown of Ssrp in the fly eye using a UAS-Ssrp<sup>RNAi</sup> transgene caused severe defects (Fig. 4D-F); supplementary material Fig. S6B,B'). Male pharate adults failed to eclose, and lacked eyes, antennae and head capsules, whereas 5.5% of females hatched, and displayed partial head capsules with absent or small eyes. Consistently, eye-antennal imaginal discs of third instar larvae were significantly smaller. Within the eye field, probably because of incomplete knockdown, few cells were phospho-Histone H3 (PH3) positive, and thus mitotically active, and few expressed Elav and mAb24B10 as indicators of photoreceptor (R-cell) differentiation. Upon overexpression of zebrafish full-length ssrp1b, full-length ssrp1a, ssrp1a<sup>as819</sup> or HMGB-deficient ssrp1a<sup>1-518</sup>, males and females hatched with fully developed heads and eyes, and proliferation and differentiation of eye-antennal imaginal discs proceeded normally (supplementary material Figs S5-S7 and Table S1). Strikingly, when Drosophila Ssrp<sup>RNAi</sup> was co-expressed with zebrafish ssrp1b, ssrp1a or ssrp1a<sup>1-518</sup>, the defects caused by Ssrp knockdown were substantially rescued (Fig. 4G-L); supplementary material Figs S6, S7). The majority of adult males and females hatched and displayed normal heads and eyes (supplementary material Table S1), apart from males rescued by ssrp1b and ssrp1a<sup>1-518</sup>, which exhibited slightly rough eyes. Proliferation and R-cell differentiation in eye-antennal discs were indistinguishable from controls. Hence, zebrafish ssrp1b, ssrp1a and ssrp1a<sup>1-518</sup> can substitute for Drosophila Ssrp. By contrast, truncated ssrp1a<sup>as819</sup> did not rescue Drosophila Ssrp knockdown phenotypes (Fig. 4M-O); supplementary material Fig. S6H,H' and Table S1), indicating that it is neither functional nor acts as a dominant-negative fragment. Importantly, these findings argue that in zebrafish, Ssrp1b could perform all Ssrp1 functions, but probably fails to replace zygotic Ssrp1a owing to low expression levels. Moreover, because Ssrp1a<sup>1-518</sup> can replace Drosophila Ssrp, our data indicate that the C-terminal HMGB-domain is dispensable in vivo. This is consistent with similar observations in vitro (Abe et al., 2011), and the fact that the yeast homologue Pob3 lacking an endogenous HMGB-domain interacts with the HMGB proteins Nhp6a and Nph6b (Wittmeyer et al., 1999). Also, other proteins can compensate for their function, as yeast lacking both factors are viable (Costigan et al., 1994). Likewise, Ssrp1a<sup>1-518</sup> could interact with other HMGB proteins to carry out FACT functions, as zebrafish and Drosophila genomes each contain at least four HMGB-containing polypeptides (Ragab et al., 2006) (Ensembl Zv9; http://www.ensembl.org/Danio rerio/Info/Index). Our in vivo findings suggest that, despite the presence of an endogenous HMGB domain, Drosophila, and probably metazoans in general, have maintained the molecular components for Pob3-like FACT function. Hence, the basic mechanism of histone reorganisation between unicellular and multicellular organisms may be conserved independently of gene fusion/separation events associated with Ssrp1 evolution.

Ssrp1a has essential functions in basic cellular processes, including DNA synthesis and gene transcription. We propose that

---

**Fig. 4. ssrp1b, ssrp1a and ssrp1a<sup>1-518</sup> substitute Drosophila Ssrp function in the eye.** (A-R') For full genotypes and sample numbers, see supplementary material Table S1. (A,D,G,J,M,P) Scanning electron micrographs of leftward-facing adult female Drosophila heads. (B,E,H,K,N,Q) Confocal images of female third instar larval eye-antennal discs (ed) and brains (br) labelled with anti-PKC (blue). GFP signals indicate the areas of transgene expression, and V5 labelling (red) the presence of Ssrp1b-V5, Ssrp1a-V5, Ssrp1a<sup>as819</sup>-V5 or Ssrp1a<sup>1-518</sup>-V5. Anterior is up. (C-C') (A-C') Controls show adult eyes with a regular array of ommatidia and eye-imaginal discs with fully developed heads and eyes, and expression of two mitotic waves (arrowheads). (A-C') Controls show adult eyes with a regular array of ommatidia and eye-imaginal discs with differentiated R-cells and two waves of dividing cells. (D-F') Drosophila Ssrp knockdown causes the loss of adult eyes, and eye-antennal discs are significantly smaller (asterisk in E). (G-R') ssrp1b (G-M'), ssrp1a (J-L') and ssrp1a<sup>1-518</sup> (P-R') expression rescues eye defects caused by Ssrp<sup>RNAi</sup>. Expression of ssrp1a<sup>as819</sup> (M-O') fails to rescue. Asterisk in N indicates eye-antennal disc defects. (F,O) Dashed lines outline eye-antennal discs.
the spatiotemporal regulation of ssr1a expression and the aforementioned functions are pivotal for achieving the appropriate rate of cell division and growth of each tissue, ensuring the correct shape, size and organ proportions in the developing embryo. Three lines of evidence indicate that maternal and zygotic Ssr1a function in a specific rather than ubiquitous fashion in zebrafish embryogenesis. First, the onset of zygotic defects in ssr1a mutants is tissue specific. Phenotypes in the eye precede those in the liver but at the same stage no phenotypes are apparent in the somites, despite an earlier phase of extensive proliferation (Sugiyama et al., 2009), suggesting that these dynamics cannot be explained solely by protein turnover associated with replication. This is consistent with specific requirements for Ssr1a in plant development (Lolas et al., 2010). Second, zygotic ssr1a-expression domains are dynamic over time and often spatially restricted, including presumptive progenitor populations. This is in line with histone chaperones performing functions central to the properties of progenitor populations. Indeed, zebrafish with germ cells lacking maternal and zygotic Ssr1a develop into sterile males, indicative of essential Ssr1a functions in germ cell formation (K.K. and A.O., unpublished). Third, the developmental defects in ssr1a mutants differ significantly in their timely appearance and severity from those in mutants carrying lesions in other genes performing similar fundamental cellular functions (Ryu et al., 2005).

In summary, our study uncovers essential functions of Ssr1a in ensuring coordinated replication and RNA transcription in vertebrate embryos, underlining the complex interplay between chromatin state and gene expression programmes during organ differentiation and growth.

Acknowledgements
We thank the NMR aquatics team for fish care; D. Stainer, H. Field and P. Dong for joining forces for the LiveEuriscreen; and A. Miyawaki, A. Sakae-Sawano, J. Fischer, T. Hawkins, K. Gassiotis, D. Wilkinson, the Bloomington Drosophila Stock Center and the DSHB (University of Iowa) for reagents or discussions.

Funding
This work was funded by the Medical Research Council [U117581329 to K.K., D.S. and E.A.O.; and U117581332 to H.A. and I.S.] Deposited in PMC for release after 6 months.

Competing interests statement
The authors declare no competing financial interests.

Supplementary material
Supplementary material available online at http://dev.biologists.orglookup/suppl/doi:10.1242/dev.093583/-/DC1

References


