Mechanisms of Development 122 (2005) 1087–1093

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Role of mitochondrial ribosome-dependent translation in germline formation in *Drosophila* embryos

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Received 5 January 2005; received in revised form 29 March 2005; accepted 13 June 2005
Available online 7 July 2005

Abstract

In *Drosophila*, mitochondrially encoded ribosomal RNAs (mtrRNAs) form mitochondrial-type ribosomes on the polar granules, distinctive organelles of the germ plasm. Since a reduction in the amount of mtrRNA results in the failure of embryos to produce germline progenitors, or pole cells, it has been proposed that translation by mitochondrial-type ribosomes is required for germline formation. Here, we report that injection of kasugamycin (KA) and chloramphenicol (CH), inhibitors for prokaryotic-type translation, disrupted pole cell formation in early embryos. The number of mitochondrial-type ribosomes on polar granules was significantly decreased by KA treatment, as shown by electron microscopy. In contrast, ribosomes in the mitochondria and mitochondrial activity were unaffected by KA and CH. We further found that injection of KA and CH impairs production of Germ cell-less (Gcl) protein, which is required for pole cell formation. The above observations suggest that mitochondrial-type translation is required for pole cell formation, and Gcl is a probable candidate for the protein produced by this translation system.

Keywords: *Drosophila*; Pole cell; Germ line; Mitochondria; Mitochndrial ribosomal RNA; Kasugamycin; Chloramphenicol; *Germ cell-less*

1. Introduction

In many animals, the factors required for germline formation are thought to be localized to the germ plasm, a histologically distinct region of the egg cytoplasm (Beam and Kessel, 1974; Eddy, 1975). Ultrastructural studies have shown that the germ plasm is basically composed of germinal granules and mitochondria (Beam and Kessel, 1974; Eddy, 1975). The germinal granules are electron-dense structures that act as a repository for the factors required for germline formation. In *Drosophila*, assembly of the germinal granules, or polar granules, requires the function of maternal effect genes. Among these are *oskar (osk), vasa (vas)*, and *tudor (tud)*, which are all essential for the formation of pole cells, the germline progenitors (Williamson and Lehmann, 1996; Mahowald, 2001; Starz-Gaiano and Lehmann, 2001). These genes produce proteins that localize to the polar granules (Hay et al., 1988; Bardsley et al., 1993; Breitwieser et al., 1996), with the association of these proteins with polar granules occurring in a stepwise and hierarchical manner (Williamson and Lehmann, 1996; Mahowald, 2001; Starz-Gaiano and Lehmann, 2001). Polar granule assembly is completed with the localization of various types of RNA to the granules. For example, mitochondrial ribosomal RNAs (mtrRNAs) and *germ cell-less* (*gcl*) mRNA are localized to the granules by *osk*, *vas*, and *tud* (Jongens et al., 1992; Kobayashi et al., 1993; Amikura et al., 1996, 2001a; Kashikawa et al., 1999).

We previously reported that mitochondrial large rRNAs (mtlrRNAs) and small rRNA (mtsrRNA) are both transported from the mitochondria to the polar granules during early embryogenesis, when mitochondria are tightly

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0925-4773/$ - see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mod.2005.06.003

associated with polar granules in the germ plasm (Akiyama and Okada, 1992; Kobayashi et al., 1993; Kashikawa et al., 1999). Together with mitochondrial ribosomal proteins, mtrRNAs form mitochondrial-type ribosomes, which are integrated into polysomes on the surface of polar granules (Amikura et al., 2001b). The mitochondrial-type ribosomes remain on the granules until pole cell formation, at which point the ribosomes and mtrRNAs are no longer discernible (Amikura et al., 1996, 2001b). Since a reduction in the amount of extra-mitochondrial mtlrRNA results in the failure to form pole cells (Iida and Kobayashi, 1998) and injection of mtlrRNA can restore pole cell formation in UV-irradiated embryos (Kobayashi and Okada, 1989), we speculate that mitochondrial-type ribosomes localized to polar granules are specifically required to produce proteins for pole cell formation.

Here, we report that inhibitors of mitochondrial (prokaryotic)-type translation, kasugamycin (KA) and chloramphenicol (CH), suppress pole cell formation when injected into early embryos. KA treatment significantly decreases the number of mitochondrial but not cytosolic ribosomes around polar granules. In contrast, mitochondrial activity and ribosomes within mitochondria are unaffected by KA and CH treatment. We further show that *gcl* mRNA is present on the periphery of the polar granules at the initiation of translation, and that KA and CH treatment represses the production of Gcl protein. Taken together, these results suggest that mitochondrial-type translation on polar granules is necessary for the production of proteins involved in pole cell formation, such as Gcl.

2. Results and discussion

To determine whether mitochondrial-type translation is required for pole cell formation, we injected KA and CH into polar plasm of early cleavage embryos at stage 2 [stages according to Campos-Ortega and Hartenstein (1997)] when

Table 1
Pole cell formation is impaired by injection of KA and CH

Injected materials[a]	No. of blastodermal embryos		
	Total	Without pole cells[b] (%)	Significance[c]
Kasugamycin	205	70 (34)	
DW	175	5 (4)	$P<0.001$
Chloramphenicol	366	134 (37)	$P<0.001$
5% Ethanol	175	6 (3)	

[a] Twenty milligram per milliliter of Kasugamycin in DW and 10 mg/ml of Chloramphenicol in 5% ethanol were injected into stage-2 embryos.
[b] The injected embryos were allowed to develop up to stage 5/6, and their pole cell formation was examined. We found that some embryos carried only a small number of pole cells (1–5), while normal embryos have 30–40 pole cells at stage 5–6. Embryos with fewer than five pole cells were judged to be 'embryos without pole cells'.
[c] Probability was calculated by the chi-square test.

Table 2
Number of Pole cells formed in the embryos injected with KA and CH

Injected materials[a]	No. of embryos examined[b]	Average number of pole cells in an embryo ± SD	Significance[c]
Kasugamycin	50	15.2 ± 7.3	
DW	32	28.4 ± 5.6	$P<0.001$
Chloramphenicol	49	20.6 ± 8.1	$P<0.001$
5% Ethanol	36	29.7 ± 5.9	

[a] Twenty milligram per milliliter of Kasugamycin in DW and 10 mg/ml of Chloramphenicol in 5% ethanol were injected into stage-2 embryos.
[b] The injected embryos were allowed to develop up to stage 4/5, and number of pole cells was counted.
[c] Probability was calculated by the *t*-test.

mitochondrial-type of ribosomes are integrated into polar granule polysomes (Amikura et al., 2001b). KA and CH are known to inhibit initiation and elongation steps of prokaryotic-type translation, respectively (Poldermans et al., 1979; Nierhaus and Wittmann, 1980). The embryos were allowed to develop until stage 6, and then pole cell formation was examined. As shown in Tables 1 and 2, pole cell formation was significantly affected by injecting KA and CH, while somatic cell formation was intact (Fig. 1B). The embryos without pole cells developed into agametic flies (data not shown).

It could be argued that KA and CH treatment might result in a decrease in mitochondrial activity, which in turn might cause defects in pole cell formation. In order to test this possibility, we stained KA- and CH-treated embryos at stage 3/4 with Rhodamine 123, a vital dye that stains active mitochondria in living cells (Johnson et al., 1980). Mitochondrial staining appeared to be largely unaffected by KA and CH treatment at the doses we used (Fig. 1D). The above result suggests that KA and CH disrupt pole cell formation without affecting mitochondrial activity.

Next, we examined the effect of KA treatment on polar granule polysomes. Since KA is known to inhibit the initiation step of prokaryotic translation (Poldermans et al., 1979), we expected that KA treatment would eliminate mitochondrial-type ribosomes from the polysomes. Although, there is no direct evidence showing that KA inhibits mitochondrial translation, the stem-loop structure in the 3′-region of small rRNA and its modification responsible for KA sensitivity are conserved in bacteria and human mitochondria (Seidel-Rogol et al., 2003). This suggests that KA is able to inhibit mitochondrial translation as well as prokaryotic one. As shown in Fig. 2, the number of mitochondrial-type ribosomes was significantly reduced by KA treatment, while the number of cytoplasmic ribosomes was unaffected. In contrast, KA did not affect ribosomes within mitochondria, consistent with the observation that mitochondrial activity was largely intact in KA-treated embryos (Fig. 1D). The average number of ribosomes in mitochondria was 151/μm^2 in KA-treated embryos, and 103/μm^2 in control embryos.