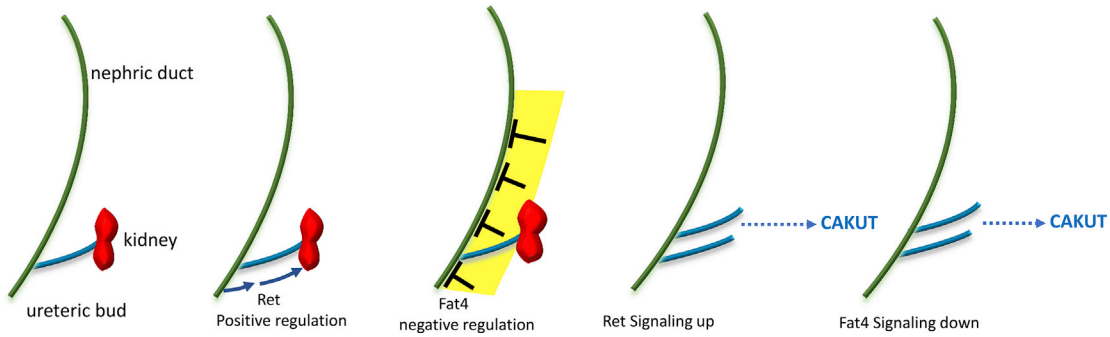

Figure 1. Proper Ureteric Bud Outgrowth Depends on Stromal-Epithelial Signaling Mediated by Fat4 and Ret
The Nephric duct has the capacity to produce epithelial sprouts along the A-P axis. The number and location of ureteric bud branches that form during development of the metanephric kidney depends on positive signals that promote sprouting (Ret/Gdnf) and negative signals such as Fat4 that restrict sprouting.

signaling via direct interaction of their respective cadherin repeats, which are closely related, and they show that these interactions are critical for restricting ureteric budding and branching to a single site at the caudal aspect of the nephric duct. The results of Zhang et al. (2019) provide a new model of stromal-ureteric bud signaling (Figure 1), regulated by Fat4 and Ret, that is critical for urinary tract formation and provide a signaling pathway that is important for modulating stereotypical ureteric bud outgrowth, an event that is central to urinary tract function. It will be interesting to determine whether *Fat4* mutations are also present in other syndromes characterized by lower urinary tract defects and whether these mutations affect Fat4's ability to interact with Ret.

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Still Searching for Specialized Ribosomes

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https://doi.org/10.1016/j.devcel.2019.03.005

Reporting in *Developmental Cell*, Cenik et al. (2019) show that the maternal ribosome supply is sufficient for *C. elegans* embryonic development, arguing against tissue-specific specialized ribosomes in this context. Examination of ribosomal requirement with the genetic tool kit presented in Artiles et al. (2019) suggests a checkpoint that prevents uncoordinated growth.

Almost as soon as the function of protein synthesis was assigned to the ribosome, the idea of ribosomes with specialized functions was suggested (Crick, 1959). This notion has periodically phased in and out of fashion and is currently a topic of intense debate (Genuth and Barna, 2018; Mills and Green, 2017). Naturally occurring ribosome heterogeneity is now well-documented, fulfilling a critical prerequisite for the existence of specialized ribosomes, and a recent report using a bacterial system (Kurylo et al., 2018) appears to have come close to meeting the biochemical benchmarks required by the

definition of specialized ribosomes (Dinman, 2016).

The complex developmental programs involved in metazoan body patterning are an attractive place to look for evidence of ribosomes with specialized functions. Quantification of mRNAs encoding ribosomal proteins (RPs) has revealed surprising variation between tissues, but it is unclear to what extent this alone creates protein-level heterogeneity in ribosomes. For example, intracellular RP transcript variation may reflect homeostatic responses to tissue-specific demands. Some evidence does exist for truly tissue-specific RP paralogs (e.g., Sugihara et al., 2010), but it is unclear whether this is essential or merely tolerated variation. More provocative are reports showing that specific, non-essential ribosomal proteins are required for proper expression of homeobox proteins involved in mouse skeletal formation (Kondrashov et al., 2011). In particular, the demonstration that these may be required for IRESs located in specific HOX mRNAs to interact with ribosomes was used to suggest that these RPs evolved for this specific function (Xue et al., 2015). However, the counterarguments, i.e., that the IRES elements evolved later to exploit these RPs as receptors, that these RPs exist in other organisms that do not undergo similar developmental processes, and the general notion of specialization through subtraction, all weaken the argument. Regardless, tissue and/or developmental stage specificity remains an appealing place to search for specialized translational machinery.

With this in mind, researchers in the Fire lab posed the question of whether any essential, tissue-specific ribosomes exist that must be produced for zygotic gene expression and report their findings in this issue of *Developmental Cell* (Cenik et al., 2019). *C. elegans*, with its extremely well-characterized developmental biology and highly sophisticated cytological, genetic, and molecular toolboxes, was exploited to look for evidence of ribosome specialization. As a first step, Cenik et al. (2019) generated a strain containing a mutation that deleted the entire cluster of ribosomal RNA genes. They also generated parallel strains that were -homozygous for null mutations in five different RP genes encoding homologs implicated in human ribosomopathies. If diverse ribosome types were produced via *de novo* production in the embryo, embryos homozygous for these rRNA or RP gene deletions may show early and/or tissue-specific defects. Unexpectedly, Cenik et al. (2019) observed that all such zygotic null RP mutants completed embryonic development normally but arrested as first-stage (L1) larvae. All tissue types examined (hypodermis, gut, cuticle, pharynx, body wall muscle, germline primordium, and neurons) showed normal phenotypic development. From these experiments, it is safe to conclude that new ribosomes need not be produced during *C. elegans* embryonic development to generate the diverse tissues of the hatchling. Importantly, the total volumes of pre-fertilization oocytes and fully differentiated L1 larvae are the same. Thus, on the basis of mass alone, maternal ribosomes are sufficient to meet the translational needs of normal embryonic processes. They also are sufficient to allow the mounting of a post-hatching heat-shock response. However, postembryonic mechanosensory neuron development and response to injury were impaired. Whether this was due to systemic signals of ribosome insufficiency (see below) or a requirement for synthesis of new, "special" ribosomes remains to be seen.

Figure 1. Still Looking for Specialized Ribosomes
Having searched for specialized ribosomes in mice, flies, yeast, bacteria, and most recently in worms, Bob casts his gaze toward *Xenopus*. Illustration by Gene Ferrick.

Cenik et al. (2019) also investigated the nature of the L1 larval arrest in mutants unable to produce zygotic ribosomes. They generated embryos in which their two founding blastomeres had distinct genotypes. This was facilitated by new genetic tools that make such mosaic analysis easier in *C. elegans*, described by Artiles et al. (2019) in an accompanying paper in this issue of *Developmental Cell*. Specifically, in the mutant embryos, the anterior (AB) blastomere is diploid but homozygous for the maternal genome, whereas the posterior (P1) blastomere becomes homozygous for the paternal genome. This was then used to create mutant larvae in which half their tissues are unable to produce new ribosomes, while the other half are normal. Given the widely held notion that nematode development is "mosaic," one might reasonably expect to see tissue-specific growth arrest combined with progression of development in wild-type tissues. However, this was not observed. Instead, development of these half mutant L1 larvae was fully arrested. In perhaps the most important insight of this study, the authors note that this suggests the existence of a body-wide checkpoint that prevents uncoordinated growth. Elucidating the nature of this checkpoint represents an important area for future work. That a similar body-wide growth arrest checkpoint does not exist in vertebrates engenders questions regarding whether or not similar intercellular crosstalk on ribosome availability occurs in other multicellular organisms, and if so, to what extent. For example, limiting this to within specific lineages or organs may explain the

tissue-specific clinical presentations and variable penetrances associated with ribosomopathies.

So, is the concept of specialized ribosomes dead? Not at all. *A priori*, the assertion that specialized ribosomes do not exist runs afoul of the basic epistemological impossibility of proving a negative. Further, at the risk of appearing facetious, specialized organelles, e.g., mitochondria and chloroplasts, certainly use ribosomes specialized for their unique biochemical environments and translational needs. But more to the point, although the authors demonstrated complete embryonic development in the absence of newly synthesized ribosomes, heterogeneity among maternal ribosomes remains a possibility because specialized organogenesis occurs during later developmental stages in these worms. However, if this is when specialization is required, then the conditions under which it matters for development become somewhat narrowed. For example, there may be body-wide use of variant ribosomes for subsets of mRNAs or translation under particular conditions, as with the IRES-containing Hox mRNAs described in mice. Such putative variants could be broadly present yet have strict tissue-specific roles because of tissue-specific transcripts. More speculatively, it is possible that there is some kind of ribosome-sorting mechanism that creates real tissue-level ribosomal heterogeneity. Direct evidence of this can only come from careful biochemical characterization of endogenous ribosomes, which is challenging in *C. elegans* due to its small size. In the end, when it comes to assigning specialized functions to "cellular" ribosomes, the challenge remains to obtain definitive evidence of their existence by biochemically matching a specific class of ribosomes to a specific class of mRNAs. The search continues (Figure 1).

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Repurposing Kinetochore Microtubule Attachment Machinery in Neurodevelopment

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https://doi.org/10.1016/j.devcel.2019.03.004

Kinetochore-microtubule attachments are essential to direct proper chromosome segregation during cell division. In this issue of *Developmental Cell*, Cheerambathur et al. (2019) and Zhao et al. (2019) uncover an unexpected role in neuronal development, unrelated to cell division, for components of the highly conserved kinetochore-microtubule attachment complex.

The KMN network is extensively studied with regard to accurate completion of mitosis. This requires sister chromatids be attached properly to spindle microtubules for the alignment and segregation of chromosomes to daughter cells. This attachment is achieved by the KMN network, a highly conserved set of protein complexes named for their major component proteins that comprised of three complexes: Knl1, Mis12, and Ndc80. This network provides the primary interface between kinetochores on centromeres and the spindle microtubules. The Knl1 complex allows recruitment of checkpoint complexes, whereas the Ndc80 complex provides the major microtubule binding interface, and the Mis12 complex recruits the Knl1 and Ndc80 complexes, thereby providing a scaffold to connect centromeric DNA to