

(15 425 predicted complete coding regions) was estimated by counting the number of matching Expressed Sequence Tags (ESTs) available in GenBank, as described previously<sup>6</sup>. This method gives only a rough estimate of gene expression level, because these ESTs have been sequenced from cDNA libraries (prepared from embryo and adult whole organisms) that have been partly normalized (Y. Kohara, unpublished). Despite this, we found a strong correlation between the number of tRNA genes and the frequency of amino-acids among *C. elegans* proteins, weighted according to their expression level (Fig. 1). As expected, this correlation is significantly stronger for highly expressed genes ( $R=0.82$ ,  $p<0.0001$ ,  $N=1631$ ) compared with those that do not match any EST (i.e. genes with very low expression level;  $R=0.61$ ,  $p=0.0042$ ,  $N=9409$ ). If the cellular tRNA abundance were not related to the number of tRNA gene, we would not expect any correlation between the frequency of amino acids and the number of tRNA genes. Therefore, these observations strongly support that in *C. elegans*, as in unicellular organisms, intracellular tRNA levels are mainly determined by gene copy number.

Interestingly, it has been noted that the tRNA gene density is higher on the X chromosome, compared with autosomes<sup>7</sup>. Indeed, there are 15.7 tRNA genes per Mb on the X chromosome, but only 3.4–4.4 tRNA genes per Mb on the autosomes – what is the reason for this? Evolutionary theory predicts that sex chromosomes evolved from an autosomal pair, and once X–Y recombination ceased, Y-linked genes were progressively inactivated and obliterated (ultimately up to the final loss of the Y-chromosome, as in *C. elegans*). As an adaptive response to the loss of Y-linked genes, homologous genes on the X-chromosome were up-regulated and subsequently subject to dosage compensation<sup>10</sup>. We propose that, in *C. elegans*, the up-expression of X-linked tRNA genes was achieved by gene duplication, which has led to the present excess of tRNA genes on the X chromosome compared with autosomes.

Gene copy numbers vary greatly among isoaccepting tRNAs. For example, among the 42 tRNA<sup>Pro</sup> genes, 32 (76%) contain the TGG anticodon (Fig. 2). Is this variation related

to a bias in codon usage? To answer this question, we computed the relative gene frequency (RGF) of each isoacceptor tRNA in the genome and the relative synonymous codon usage (RSCU) in highly expressed genes. There is a highly significant correlation between RGF and the RSCU of complementary codons ( $R=0.54$ ,  $p<0.0001$ ). It should be noticed that this correlation reflects only partially the real co-adaptation of tRNA abundance and codon usage because the same tRNA can decode several codons. Although we have no experimental data on base modifications in *C. elegans* tRNAs (except for tRNA Leu-AAG, with an inosine modification at the first anticodon position<sup>11</sup>), it is possible to predict the codons decoded by the different anticodons according to the classical rules<sup>12</sup>: (1) G–U wobble pairing; (2) an I at the first anticodon position produces a preference for U or C over A; and (3) As at the first anticodon position are predicted as Is, because an unmodified A has been found only in a few exceptional cases. This analysis revealed that all the favored codons are decoded by the isoaccepting tRNA that has the highest gene copy number (Fig. 2). In the three cases where there are two favored codons (Arg, Leu and Ala), both codons are decoded by the major isoaccepting tRNA. Moreover, in all cases where an I is predicted at the first anticodon position, favored codons end in C and/or U. Finally, for eight of the nine duets, the favored codon is the direct complement of the major isoaccepting tRNA. In conclusion, codon-usage biases and tRNA gene redundancy in *C. elegans* genome clearly reflect a co-adaptation of tRNA content and codon usage for the optimal translation of the pool of highly expressed genes.

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#### Author's correction

Deng, X-W. *et al.* (2000) Unified nomenclature for the COP9 signalosome and its subunits: an essential regulator of development. *Trends Genet.* 16, 202–203

An error occurred during the publication of the letter by Deng *et al.* in the May issue. The correct citation for this letter should be:

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