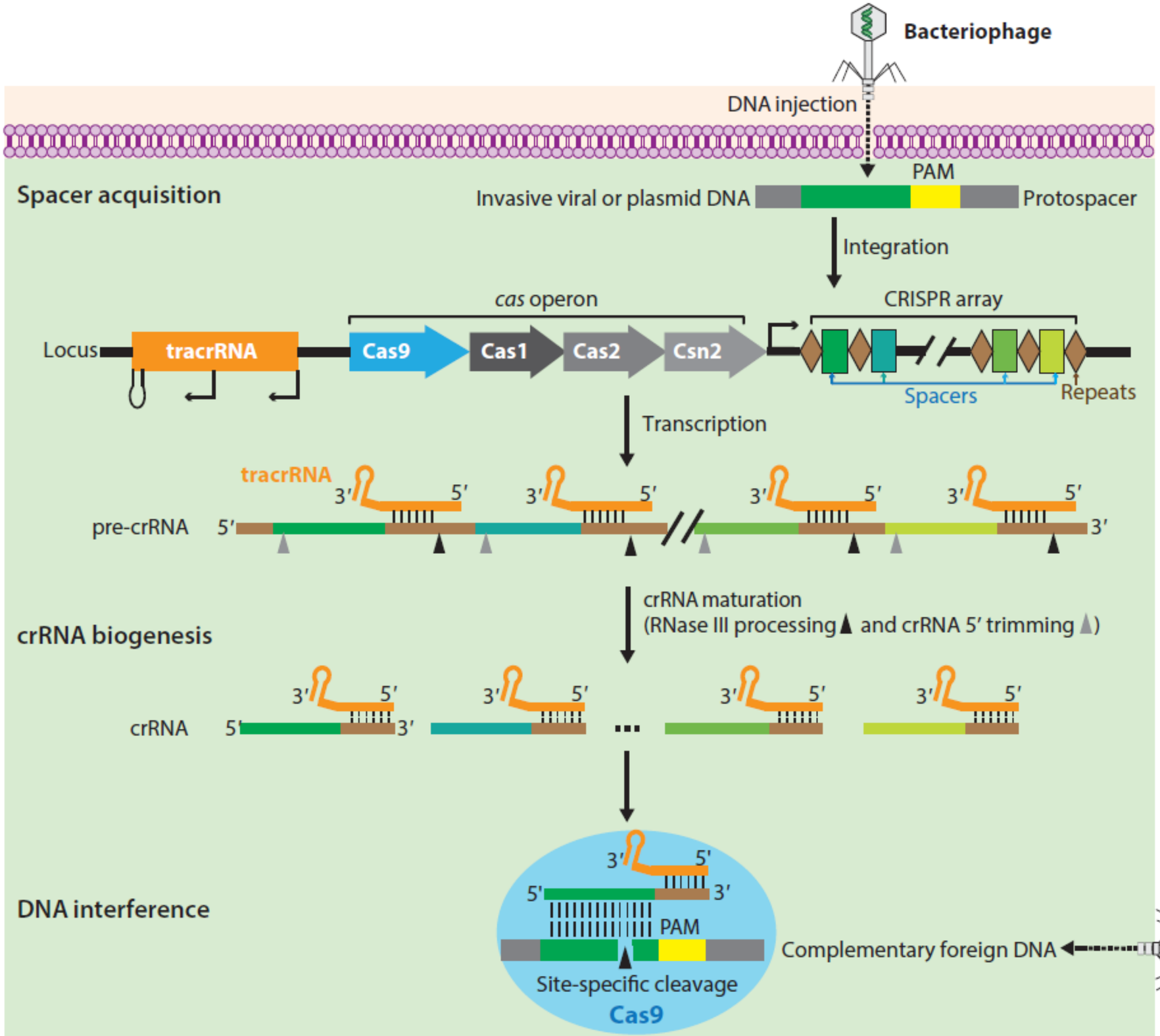

CRISPR–Cas9-mediated DNA interference in bacterial adaptive immunity

A typical CRISPR locus in a type II CRISPR–Cas system comprises an array of repetitive sequences (repeats, *brown diamonds*) interspaced by short stretches of nonrepetitive sequences (spacers, *colored boxes*), as well as a set of CRISPR-associated (*cas*) genes (*colored arrows*).

Preceding the *cas* operon is the *trans*-activating CRISPR RNA (*tracrRNA*) gene, which encodes a unique noncoding RNA with homology to the repeat sequences.

Upon phage infection, a new spacer (*dark green*) derived from the invasive genetic elements is incorporated into the CRISPR array by the acquisition machinery (Cas1, Cas2, and Csn2).

Once integrated, the new spacer is cotranscribed with all other spacers into a long precursor CRISPR RNA (pre-crRNA) containing repeats (*brown lines*) and spacers (*dark green*, *blue*, *light green*, *and yellow lines*).

The tracrRNA is transcribed separately and then anneals to the pre-crRNA repeats for crRNA maturation by RNase III cleavage.

Further trimming of the 5′ end of the crRNA (*gray arrowheads*) by unknown nucleases reduces the length of the guide sequence to 20 nt.

During interference, the mature crRNA–tracrRNA structure engages Cas9 endonuclease and further directs it to cleave foreign DNA containing a 20-nt crRNA complementary sequence preceding the PAM sequence.

From Jiang and Doudna. CRISPR-Cas9 Structures and Mechanisms. *Annu Rev Biophys*. 46:505-29; 2017