

F. C. Kafatos: RNAi Screens and their Use to Decipher Immune Response Modules in *Anopheles gambiae*

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Our laboratory group has introduced direct injection of dsRNA as an effective mode of RNAi-based gene silencing in adult mosquitoes and reverse genetics (Blandin *et al.*, 2001).

The group has now performed in depth analysis of a number of genes as well as several gene screens (some in collaboration with E. Levashina) permitting us to make some general statements about this knockdown (KD) procedure.

- It has wide applicability as evidenced by KD studies of well over 100 genes to date. A 4-day exposure to injected dsRNA is sufficient in most cases; we anticipated that genes for very stable proteins may require prolonged dsRNA treatment.
- The procedure is applicable to at least three tissues (fat body, hemocytes, midgut); no effort has been made to test effects on sequestered tissues such as the nervous system.
- Inevitably, injection engenders a physical challenge with potentially disseminatable effects; however these and any potential effects of dsRNA *per se* can be subtracted by including controls in each experiment (e.g. *dsGFP* or *dslacZ*).
- It is advisable to confirm gene silencing by QRT-PCR and if antibodies are available, by immunoblotting; typically the RNA-level assays underestimate silencing, which is best assessed at the protein level.
- There is sufficient variability to require vigorous statistical assessment of phenotypes; typically, we utilize equal numbers of experimental and control samples per gene (at least 40 each), and evaluate the significance of the observed effects by either the Kolmogorov–Smirnov or the Wilcoxon-two sample test.
- Simultaneous double (or possibly triple) KDs are possible, and they permit epistasis analysis and the characterization of genetic modules or pathways.

I will illustrate these features by summarizing the results of two innate immunity screens which are currently in press and one that is under review: Moita *et al.* (phagocytosis), Vlachou *et al.* (local epithelial responses) and Osta *et al.* (melanization module).