RNA INTERFERENCE AS A TOOL FOR CHICKEN FUNCTIONAL GENOMICS

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SUMMARY
Functional analysis of newly identified genes has become a major part of genomic projects and will obviously become important for gene discovery and annotation of the recently sequenced chicken genome. Mutagenesis and transgenic approaches have become routine for functional genomic studies in species such as mice (Okabe *et al*., 1997), zebrafish (Gong *et al*., 2002) and drosophila (Link, 2002), however these techniques are still being developed for chickens and to date remain far from routine. A recent phenomenon known as RNA interference (RNAi) promises to advance the elucidation of gene function in chicken cells. We have evaluated and demonstrated the knockdown potential of both siRNA and shRNA molecules in the chicken DF1 cell line.
Keywords: RNAi, gene silencing, functional genomics

INTRODUCTION
RNAi is a method of sequence specific gene knockdown and has become an incredibly useful tool to analyse gene function in both plants and animals (Napoli *et al*., 1990; Sharp, 1999; Elbashir *et al*., 2001). RNAi describes the post-transcriptional silencing of gene expression in response to the introduction of double-stranded RNA (dsRNA) into cells. The conserved RNAi pathway involves the processing of dsRNA duplexes into 21-23 nucleotide (nt) molecules known as small interfering RNAs (siRNA) to initiate gene knockdown (Fire, 1999; Bosher and Labouesse, 2000; Hannon, 2002). Since the discovery of RNAi in animals (Fire et al., 1999) the use of long dsRNA in lower eukaryotes, especially in the model organism *Caenorhabditis elegans*, has been used to determine gene function (Barstead, 2001; Ashrafi *et al*., 2003). However, in vertebrate systems the cellular uptake of long dsRNA induces an antiviral defence mechanism initiated by interferon (IFN), leading to non-specific translational shutdown and apoptosis (Williams, 1997; Stark *et al*., 1998; Gil and Esteban, 2000). This non-specific cellular activity can be circumvented by the direct transfection of either chemically synthesised or *in vitro* transcribed siRNAs of approximately 21 nt in length into mammalian cells (Tuschl, 2002). These short molecules do not activate the IFN response, but can induce reliable and efficient transient knockdown of target genes (Dykxhoorn *et al*., 2003; Duxbury and Whang, 2004). As a consequence, the development of DNA-based vectors for expression of short hairpin RNA (shRNA) molecules that are processed within the cell to produce active siRNA molecules have been developed (Brummelkamp *et al*., 2002; Yu *et al*., 2002; Paddison *et al*., 2002). Such DNA expression constructs have achieved highly efficient gene knockdown without induction of the IFN response.

There are very few reports of the use of RNAi for gene function analysis in chicken cells, therefore we have evaluated and demonstrated the knockdown potential of both siRNA and shRNA molecules in the chicken DF1 cell line. We used a plasmid (pEGFP–N1, Clontech) encoding enhanced green fluorescent protein (EGFP) as the target gene for RNAi.