



Eukaryotic transcriptional control

Roger D. Kornberg

Some 30 years ago, following the elucidation of transcriptional control in prokaryotes, attention turned to the corresponding problem in eukaryotes: how are so many genes transcribed in a cell-type-specific, developmentally regulated manner? The answer has been found in two modes of regulation, one involving chromatin and the other the chief transcribing enzyme, RNA polymerase II. Although basic features of the prokaryotic mechanism have been preserved, the demands of eukaryotic transcription control are met by a huge increase in complexity and by the addition of new layers to the transcription apparatus. Discovering the components of this apparatus has been a major theme of research over the past three decades; unravelling the mechanisms is a challenge for the future.

Many molecules involved in eukaryotic transcription and its control have now been identified, including nucleosomes, polymerases, general transcription factors and the regulatory apparatus. Recent excitement in the field stems, in part, from two findings. First, transcriptional coactivators and corepressors function by enzymatic modification of histones to modulate repression by nucleosomes. Second, Mediator, first defined in yeast, is an important basis of regulation by nuclear receptors and other transcription control proteins in mammalian cells. Additional key insights into the transcription mechanism have come from studies of prokaryotic RNA polymerases.

Repression by nucleosomes

Coiling of DNA around a histone octamer in the nucleosome is now recognized as a cornerstone of transcriptional control^{1,2}. Nucleosomes repress all genes except those whose transcription is brought about by specific positive regulatory mechanisms: they establish a zero baseline above which eukaryotic transcriptional control can be observed.

Negative regulation in prokaryotes is effected by gene-specific repressors and occurs at a minority of promoters. This mechanism would be ill-suited to eukaryotes, given the number of different repressors needed to extinguish the expression of most genes in most cell types. Instead, a general repression mechanism would provide a better basis for cell-type-specific regulation of the large eukaryote genomes.

Nucleosomes repress transcription in at least three different ways. First, they occlude sites of protein binding to DNA, thereby interfering with the interaction of activator and repressor proteins, polymerases and transcription factors, DNA-modifying enzymes and the like³. Second, chains of nucleosomes can become further coiled or folded, and this higher-order coiling represses transcription of entire chromosomal domains^{4,5}. Finally, interactions of nucleosomes with additional chromosomal proteins in heterochromatin repress gene expression in a hereditary manner⁶.

The structural basis of repression by nucleosomes can be traced to the histone configuration. Each histone is organized in two domains, a characteristic 'histone fold' and an unstructured N-terminal 'tail'. The histone-fold domains constrain the DNA in a central core particle and, thereby, restrict access of DNA-binding proteins. The tails extend outside the core particle, providing points of interaction for higher-order coiling, condensation in heterochromatin and gene activation.

Gene activator proteins

Early work on eukaryotic transcriptional control focused on positive regulatory DNA elements, known as enhancers, and on proteins, termed activators, that bind to them and stimulate transcription.

Delineation of enhancers by mutagenesis and detection of activators by DNA-binding analysis revealed many types. Activators are specific for genes or gene families and, typically, couple transcription to the physiological needs of the cell. Activators are often latent and become functional in transcription during a physiological response. Particularly well studied in this regard are 'signal transducer and activator of transcription' (STAT) proteins⁷, which transduce signals from growth factor and cytokine receptors, nuclear factor κ B (NF- κ B)⁸, which mediates immune and inflammatory responses, and nuclear hormone receptors⁹, which interact with a wide array of lipophilic signalling molecules. STATs are phosphorylated by receptor tyrosine kinases or by receptor-associated Janus kinases (JAKs), whereupon they dimerize, enter the nucleus and activate transcription. NF- κ B is maintained in a 'masked' state in the cytoplasm through its association with an inhibitory subunit, I κ B. Phosphorylation of I κ B by cell-surface receptors and its destruction by proteolysis releases the NF- κ B activator in a fully functional form to enter the nucleus. Finally, nuclear hormone receptors can be persistently associated with target genes and require only binding of the hormone to activate transcription.

Activator proteins are modular, with distinct DNA-binding and activation domains. The DNA-binding domains fall into several major families, whose structures are known from crystallographic or nuclear magnetic resonance (NMR) analysis¹⁰. The affinity and specificity of DNA binding is sometimes augmented, and also regulated, by interactions with accessory proteins bound to adjacent DNA sites (thereby forming what has been termed an 'enhanceosome'¹¹).

Activation domains are, by contrast, poorly understood. It is thought that they contact components of the transcription machinery to influence the initiation of the transcription reaction. Many such interactions have been described, but the chain of contacts leading to transcription remains to be established.

Gene activation: relief of repression by nucleosomes

Separate lines of work on chromatin structure and gene activator proteins recently intersected to reveal a key aspect of the activation mechanism^{1,12-17}. Proteins previously identified on the basis of interaction with activator proteins, or implicated by genetic screens in transcriptional control, were discovered to possess catalytic activities directed at the histones (Fig. 1, Table 1). In human cells, for example, the related proteins CBP [for 'cAMP-responsive-element-binding (CREB)-binding protein'] and p300 bind to activator proteins involved in cell-cycle control, differentiation, DNA repair and apoptosis; they stimulate transcription and therefore are termed 'coactivators'. CBP and p300 acetylate the histone tails, a modification characteristic of transcribed chromatin. This, and related findings, suggested a role for chromatin structure in

Roger D. Kornberg
kornberg@cellbio.
stanford.edu

Dept of Structural
Biology, Stanford
School of Medicine,
Stanford, CA 94305,
USA.

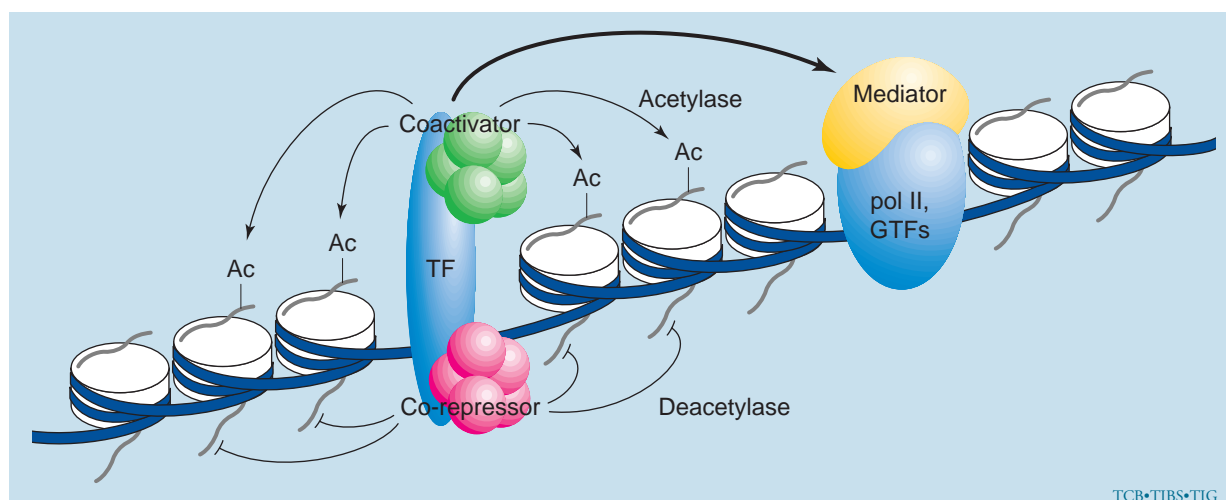


FIGURE 1. Two modes of regulation of eukaryotic gene transcription are depicted schematically, with a transcription factor (TF; blue), either an activator or a repressor, bound to a regulatory element, and RNA polymerase II (pol II; blue) and general transcription factors (GTFs; blue) bound to a promoter, along the DNA (dark blue ribbon). Histone octamers are drawn as disks, with protruding histone tails (grey wavy lines). An activator stimulates transcription both by interaction with a coactivator (green), which recruits an acetylase to acetylate (Ac) the histone tails, and by direct interaction with Mediator (yellow). A repressor interacts with a co-repressor (pink), which recruits a histone deacetylase.

TCB•TIBS•TIG

transcriptional activation. As the histone tails lie outside the core particle of the nucleosome and do not contribute to its organization or stability, acetylation is more likely to affect higher-order chromatin structure. A reasonable inference would be that activators stimulate transcription, in part, by relief of repression owing to the higher-order structure.

Coincident with the histone acetyltransferase activities of coactivators was the discovery of histone deacetylase activities of co-repressors. Much as acetylation can relieve repression by chromatin, deacetylation can re-establish repression. Histone deacetylation might also explain the long-standing correlation between DNA methylation and lack of gene expression: a protein that binds to methylated DNA recruits a multiprotein histone-deacetylase complex¹⁸. The mechanism of genomic imprinting, whereby the expression level of a gene depends on its parental origin, is believed to entail DNA methylation and, thus, the establishment of a repressed structure of chromatin.

Despite the importance of histone acetylation, this is not sufficient for transcriptional activation as it fails to disrupt the core particle of the nucleosome. The inhibition of polymerase and transcription factor binding to DNA within core particles must be relieved by alternative means. This alternative probably is provided by multiprotein complexes that 'remodel' the structure of chromatin in an ATP-dependent manner. Two families of such complexes, termed SWI/SNF and ISWI (for 'imitation switch'), can be distinguished on the basis of their ATPase components. Members of the SWI/SNF family perturb the core-particle structure, as shown by increased susceptibility of the DNA to nuclease attack, whereas ISWI complexes are especially proficient at shifting the locations of nucleosomes on DNA. Both types of complex might contribute to the formation of apparently nucleosome-free regions, known as 'nuclease-hypersensitive sites', which contain the enhancers and promoters of transcriptionally active genes. Exposure of an enhancer is presumably important early in gene activation, which could explain why the SWI/SNF complex is the first coactivator to arrive at a locus undergoing the induction of transcription (in yeast)¹⁹.

Gene activation: Mediator complex

The initiation of transcription proceeds in two stages: relief of repression by chromatin, followed by the interaction of polymerase

and accessory factors with the promoter. Activator proteins are presumed to trigger the first stage by recruiting chromatin-modifying complexes. How activators might stimulate the second stage has been the subject of debate, only recently resolved by the convergence of studies in yeast and human systems on a common mechanism^{20,21}. With this development, a long-term effort to reconstitute correctly regulated transcription initiation with purified proteins has finally been achieved.

Work along these lines began with the discovery of the three eukaryotic RNA polymerases, designated I, II and III (Ref. 22),

TABLE 1. RNA polymerase II transcription machinery^a

	Number of subunits	Catalytic subunit(s)
Histone acetyltransferase^{b,c}		
CBP/p300 (<i>H.s.</i>)	1	
SAGA (<i>S.c.</i>)	≥14	Gcn5p
PCAF (<i>H.s.</i>)	~20	PCAF
Elongator (<i>S.c.</i>)	3	Elp3p
Histone deacetylase^{b,c}		
mSin3 (<i>H.s.</i>)	~10	HDAC1, HDAC2
NURD (<i>H.s.</i>)	~18	HDAC1, HDAC2 (also CHD3, CHD4 ATPases)
Chromatin-remodelling complex^{b,c}		
SWI/SNF (<i>S.c.</i>)	11	Swi2p/Snf2p (ATPase)
NURF (<i>D.m.</i>)	4	ISWI (ATPase)
RNA polymerase II	12	
General transcription factor^c		
TFIIB	1	
TFIID	9	
TFIIE	2	
TFIIF	2	
TFIIH (<i>S.c./H.s.</i>)	9	Sst2p/Xpb (helicase), Rad3p/Xpd (helicase), Kin28p (kinase)
Mediator	20	

^aRefs 1, 3, 25 and 42 feature reviews or articles that highlight the RNA polymerase II transcription machinery.

^bExamples of histone acetyltransferase, histone deacetylase and chromatin-remodelling complexes are listed. Many additional members of these categories are known.

^cAbbreviations: *D.m.*, *Drosophila melanogaster*; *H.s.*, *Homo sapiens*; *S.c.*, *Saccharomyces cerevisiae*.

and the recognition of their structural and functional complexity^{23,24}. Most attention has been directed towards RNA polymerase II, responsible for all messenger RNA (mRNA) synthesis, although important insights have also come from work on polymerases I and III, which transcribe the genes for ribosomal RNA (rRNA) and transfer RNA (tRNA), respectively. The polymerases are large multiprotein complexes, which require further proteins, termed general transcription factors, to recognize a promoter and initiate transcription. The general transcription factors for polymerase II, designated TFIIB, -D, -E, -F and -H, were first isolated from yeast, rat and human cells²⁵. The complete set of factors comprises 23 polypeptides, which, along with the 12 subunits of the polymerase, show a perfect one-to-one correspondence between yeast and human²⁶.

The TFIID complex is responsible for promoter recognition²⁷. The TATA-binding protein (TBP) subunit of the complex binds to the TATA box, whereas the remaining subunits, known as TBP-associated factors (TAFs), interact with flanking sequences and confer additional specificity²⁸. Bending of TATA-box DNA around TBP creates a context for interaction with TFIIB, which, in turn, positions the promoter on the polymerase^{29,30}. TFIIF includes ATP-dependent helicases that unwind the promoter around the start site to trigger the initiation of transcription³¹.

Gene activator proteins bind to TFIIB, TFIID and TFIIF. Genetic studies implicate these interactions in gene activation *in vivo*. Activator proteins fail, however, to stimulate transcription reconstituted with polymerase II and general transcription factors *in vitro*. Therefore, an additional protein factor is required³², termed 'Mediator'. Isolated from yeast as a 20-subunit complex, Mediator was shown to interact with polymerase II, forming a 'holoenzyme' complex^{33,34}. Two-thirds of the Mediator subunits are products of genes previously identified in disparate screens for mutations that affect transcription control in yeast³⁵, demonstrating the wide significance of Mediator for regulation *in vivo*. The genetically defined subunits include the suppressor of RNA polymerase B (Srb) proteins, one of which, when mutated, abolishes almost all transcription *in vivo*³⁶.

A key question as to whether the Mediator mechanism also applied to higher organisms was answered by the recent isolation of corresponding complexes from mouse and human cells (Ref. 37 and references therein). These complexes varied somewhat in subunit composition, but recent evidence emphasizes their similarity. The human Mediator complex had, in fact, been isolated previously by virtue of its association with ligand-bound, but not unliganded, nuclear receptors, providing direct evidence of activator-Mediator interaction³⁸. Mediator is now recognized as an important interface between activators and polymerase II. It transduces regulatory information from enhancers to promoters in organisms ranging from yeast to human.

Transcription elongation: lessons from prokaryotes

Activators not only regulate transcription initiation by the relief of repression and through Mediator but they also appear to stimulate the subsequent elongation process³⁹. Most of what is known

about transcription elongation derives from studies of bacterial RNA polymerase (Ref. 40 and references therein). Although the bacterial enzyme is much simpler than RNA polymerase II, possessing only four rather than 12 types of subunit, the two largest contain regions of similarity. A key feature of the regulation of elongation by the bacterial polymerase is its capacity to oscillate between forward and backward movement at every residue of the template. Studies of the bacterial polymerase have revealed other important, conserved aspects, such as the existence of a 'bubble' of unwound DNA around the nucleotide polymerization site, and the association of the RNA product with the template through base-pairing of about eight residues immediately adjacent to the polymerization site.

Outstanding issues

Two major themes prevail: (1) the relief by modifying enzymes and remodelling factors of nucleosome-induced repression; and (2) the central role of Mediator in the transduction of regulatory information from enhancers to promoters. Current and future research will no doubt address some of the issues discussed below.

The molecular mechanism of Mediator action

At first, it was thought that activators might function by 'recruitment' of general transcription factors to promoters, analogous to the mechanism of positive control in prokaryotes. Then, it emerged that activators make crucial contacts with Mediator, whose influence on RNA polymerase and the initiation reaction have yet to be determined. This issue subsumes a host of questions concerning the activation mechanism. What is the relative importance of activator-Mediator and activator-general transcription factor (including activator-TFIID) interactions? Do different activators contact different subunits of Mediator, and is the subunit composition constant or does it vary among cell types? How do some subunits of Mediator function in both positive and negative regulation, as shown by genetic analysis in yeast?

The role of chromatin structure beyond the nucleosome

Little is known about higher-order structure and its involvement in transcriptional regulation. It might underlie the coordinate regulation of multiple genes within large chromosomal domains. DNA elements responsible for such regulation include 'locus control regions'⁴¹, which resemble enhancers, and 'insulators'⁵, which define the boundaries of a domain. Beyond their characterization by molecular genetics, the mode of action of these elements remains obscure.

Epigenetic regulation of gene expression, as in gene silencing by heterochromatin

Recent studies in yeast have shown that heterochromatin is formed by the interaction of silent information regulator (SIR) proteins with the tails of histones H3 and H4 protruding from nucleosomes⁶. Understanding the mechanisms by which heterochromatin spreads along a chromosome and is inherited at cell division await the determination of its structure.

References

- Kornberg, R.D. and Lorch, Y. (1999) Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell* 98, 285–294
- Struhl, K. (1999) Fundamentally different logic of gene regulation in eukaryotes and prokaryotes. *Cell* 98, 1–4
- Workman, J.L. and Kingston, R.E. (1998) Alteration of nucleosome structure as a mechanism of transcriptional regulation. *Annu. Rev. Biochem.* 67, 545–579
- Ramakrishnan, V. (1997) Histone H1 and chromatin higher-order structure. *Crit. Rev. Eukaryotic Gene Expr.* 7, 215–230
- Bell, A.C. and Felsenfeld, G. (1999) Stopped at the border: boundaries and insulators. *Curr. Opin. Genet. Dev.* 9, 191–198
- Grunstein, M. (1998) Yeast heterochromatin: regulation of its assembly and inheritance by histones. *Cell* 93, 325–328
- Darnell, J.E.J. (1997) STATs and gene regulation. *Science* 277, 1630–1635
- Ghosh, S. *et al.* (1998) NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu. Rev. Immunol.* 16, 225–260
- Mangelsdorf, D.J. *et al.* (1995) The nuclear receptor superfamily: the second decade. *Cell* 83, 835–839

- 10 Harrison, S.C. (1991) A structural taxonomy of DNA-binding domains. *Nature* 353, 715–719
- 11 Maniatis, T. *et al.* (1998) Structure and function of the interferon-beta enhanceosome. *Cold Spring Harbor Symp. Quant. Biol.* 63, 609–620
- 12 Struhl, K. (1998) Histone acetylation and transcriptional regulatory mechanisms. *Genes Dev.* 12, 599–606
- 13 Gregory, P.D. and Horz, W. (1998) Life with nucleosomes: Chromatin remodelling in gene regulation. *Curr. Opin. Cell Biol.* 10, 339–345
- 14 Varga-Weisz, P.D. and Becker, P.B. (1998) Chromatin-remodeling factors: Machines that regulate? *Curr. Opin. Cell Biol.* 10, 346–353
- 15 Cairns, B.R. (1998) Chromatin remodeling machines: Similar motors, ulterior motives. *Trends Biochem. Sci.* 23, 20–29
- 16 Kadonaga, J.T. (1998) Eukaryotic transcription: An interlaced network of transcription factors and chromatin-modifying machines. *Cell* 92, 307–317
- 17 Wu, C. (1997) Chromatin remodeling and the control of gene expression. *J. Biol. Chem.* 272, 28171–28174
- 18 Ng, H.H. and Bird, A. (1999) DNA methylation and chromatin modification. *Curr. Opin. Genet. Dev.* 9, 158–163
- 19 Cosma, M.P. *et al.* (1999) Ordered recruitment of transcription and chromatin-remodelling factors to a cell-cycle and developmentally regulated promoter. *Cell* 97, 299–311
- 20 Bjorklund, S. and Kim, Y.-J. (1996) Mediator of transcriptional regulation. *Trends Biochem. Sci.* 21, 335–337
- 21 Bjorklund, S. *et al.* (1999) Global transcription regulators of eukaryotes. *Cell* 96, 759–767
- 22 Roeder, R.G. and Rutter, W.J. (1969) Multiple forms of DNA-dependent RNA polymerase in eukaryotic organisms. *Nature* 224, 234–237
- 23 Keding, C. *et al.* (1970) Alpha-amanitin: a specific inhibitor of one of two DNA-dependent RNA polymerase activities from calf thymus. *Biochem. Biophys. Res. Commun.* 38, 165–171
- 24 Sklar, V.E.F. *et al.* (1975) Distinct molecular structures of nuclear class I, II and III DNA-dependent RNA polymerases. *Proc. Natl. Acad. Sci. U. S. A.* 72, 348–352
- 25 Conaway, R. and Conaway, J. (1997) General transcription factors for RNA polymerase II. *Prog. Nucleic Acids Res. Mol. Biol.* 56, 327–346
- 26 Feaver, W.J. *et al.* (1996) Genes for Tfb2, Tfb3, and Tfb4 subunits of yeast transcription/repair factor IIH: Homology to human cyclin-dependent kinase activating kinase and IIH subunits. *J. Biol. Chem.* 272, 19319–19327
- 27 Shen, W.-C. and Green, M.R. (1997) Yeast TAF_{II}145 functions as a core promoter selectivity factor, not a general coactivator. *Cell* 90, 615–624
- 28 Burley, S.K. and Roeder, R.G. (1996) Biochemistry and structural biology of transcription factor IID (TFIID). *Annu. Rev. Biochem.* 65, 768–799
- 29 Li, Y. *et al.* (1994) RNA polymerase II initiation factor interactions and transcription start site selection. *Science* 263, 805–807
- 30 Leuther, K.K. *et al.* (1996) Two-dimensional crystallography of transcription factor IIB- and IIE-RNA polymerase II complexes: Implications for start site selection and initiation complex formation. *Cell* 85, 773–779
- 31 Svejstrup, J.Q. *et al.* (1996) The multiple roles of transcription/repair factor TFIIF. *Trends Biochem. Sci.* 21, 346–350
- 32 Flanagan, P.M. *et al.* (1991) A mediator for activation of RNA polymerase II transcription *in vitro*. *Nature* 350, 436–438
- 33 Thompson, C.M. *et al.* (1993) A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. *Cell* 73, 1361–1375
- 34 Kim, Y.-J. *et al.* (1994) A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. *Cell* 77, 599–608
- 35 Myers, L.C. *et al.* (1998) The Med proteins of yeast and their function through the RNA polymerase II C-terminal domain. *Genes Dev.* 12, 45–54
- 36 Thompson, C.M. and Young, R.A. (1995) General requirement for RNA polymerase II holoenzymes *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* 92, 4587–4590
- 37 Ito, M. *et al.* (1999) Identity between TRAP and SMCC complexes indicates novel pathways for the function of nuclear receptors and diverse mammalian activators. *Mol. Cell* 3, 361–370
- 38 Fondell, J.D. *et al.* (1996) Ligand induction of a transcriptionally active thyroid hormone receptor coactivator complex. *Proc. Natl. Acad. Sci. U. S. A.* 93, 8329–8333
- 39 Bentley, D.L. (1995) Regulation of transcriptional elongation by RNA polymerase II. *Curr. Opin. Genet. Dev.* 5, 210–216
- 40 Korzhova, N. *et al.* (1998) Mechanistic model of the elongation complex of *Escherichia coli* RNA polymerase. *Cold Spring Harbor Symp. Quant. Biol.* 63, 337–345
- 41 Grosveld, F. (1999) Activation by locus control regions? *Curr. Opin. Genet. Dev.* 9, 152–157
- 42 Kornberg, R.D. and Lorch, Y. (1999) Chromatin-modifying and -remodeling complexes. *Curr. Opin. Genet. Dev.* 9, 148–151

Death by design: mechanism and control of apoptosis

Zhiwei Song and Hermann Steller

Active cellular suicide by apoptosis plays important roles in animal development, tissue homeostasis and a wide variety of diseases, including cancer, AIDS, stroke and many neurodegenerative disorders. A central step in the execution of apoptosis is the activation of an unusual class of cysteine proteases, termed caspases, that are widely expressed as inactive zymogens. Originally, the mechanisms for regulating the caspase-based cell death programme seemed to be different in *Caenorhabditis elegans*, mammals and insects. However, recent results suggest that these apparent differences in the control of cell death reflect our incomplete knowledge, rather than genuine mechanistic differences between different organisms.

Apoptosis is a morphologically distinct form of cell death that is designed to rapidly remove unwanted and potentially dangerous cells^{1,2}. During the development of most metazoan animals, many more cells are produced than are eventually needed, and apoptosis plays a key role in removing surplus cells and sculpting the developing embryo³ (Fig. 1). In addition, the inappropriate regulation of apoptosis is associated with a variety of diseases, including cancer, AIDS, neurodegenerative diseases and ischaemic stroke⁴. Because apoptosis represents an active,

gene-directed mechanism, it should eventually be possible to control this process for therapeutic purposes.

During the past few years, rapid progress has been made in identifying some of the molecules that are responsible for the regulation and execution of apoptosis. The existence of a cell-suicide programme was originally proposed on the basis of the stereotyped, morphological changes associated with natural cell death, but definitive evidence for the existence of a designated death programme came from genetic studies of cell death in *Caenorhabditis*



Zhiwei Song
zhiwei@wccf.mit.edu

Hermann Steller
hsteller@mit.edu

Howard Hughes
Medical Institute,
Dept of Biology and
Dept of Brain and
Cognitive Sciences,
Massachusetts
Institute of
Technology, 68-430,
77 Massachusetts
Avenue, Cambridge,
MA 02139, USA.