Genomic imprinting in mammalian development: a parental tug-of-war

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Genomic imprinting in mammals is increasingly being implicated in developmental and pathological processes, but without a clear understanding of its function in normal development. We believe that imprinting has evolved in mammals because of the conflicting interests of maternal and paternal genes in relation to the transfer of nutrients from the mother to her offspring. We present an hypothesis that accounts for many of the observed effects of imprinting in mammals and relates them to similar observations in plants. This hypothesis has implications for studies of X-chromosome inactivation and a range of human diseases.

The term genomic imprinting has been used to refer to all cases where genes have differential expression depending on the sex of the parent from which they are inherited[1–3]. At least two distinct phenomena have been described as imprinting and it is important to distinguish between them. In fungus gnats (Sciaridae), gall midges (Cecidomyidae) and scale insects (Coccoidea), the paternal set of chromosomes is eliminated during spermatogenesis[4,5]. This can be understood as a special form of meiotic drive, under the control of the maternal genome. Paternal chromosomes are marked for elimination in the egg cytoplasm and do not contribute genes to the next generation[4,5]. Imprinting in mammals and flowering plants belongs in another category, because maternal and paternal genomes have different phenotypic effects during development but contribute equally to gametes at meiosis. Our paper presents a selective process that can explain the evolution of imprinting in mammals but does not address the underlying molecular mechanisms.

Of mice and maize

There are intriguing parallels between imprinting in mammals and angiosperms (flowering plants). Both groups display a division of labour during development, between the embryo proper, which gives rise to the adult body, and tissues whose principal function is the acquisition of nutrients from the mother. The acquisitive tissues are the extraembryonic membranes of mammals and the endosperm of angiosperm seeds. These tissues have very different origins (Fig. 1). The extraembryonic membranes are derived from the zygote, whereas the endosperm is derived from the fusion of two haploid maternal nuclei with a second sperm nucleus. The endosperm is genotypically identical to the embryo except that it has two copies of the maternal genome for each copy of the paternal genome.

Despite these differences, both groups display remarkably similar responses to disruption of the normal ratio of maternal to paternal genomes. Normal development of maize endosperm requires a 2m:1p ratio of maternal to paternal genomes (Fig. 2). Thus, hexaploid endosperms with two paternal genomes (4m:2p) develop normally, but hexaploid endosperms with one paternal genome (5m:1p) are abortive[6]. Several chromosome regions are suspected of being imprinted in maize. Loss of the short arm of paternal chromosome 4, or the long arm of paternal chromosome 1 or 10, results in mature endosperms that are smaller than normal. Kernel size is not restored by adding extra copies of maternally derived homologues[7,8]. Lin has mapped three endosperm growth factors that contribute to the imprinting effects of chromosome 10 (Ref. 7).

Endosperm development has been described in reciprocal crosses between diploid and autotetraploid maize. When the diploid is the seed parent, the

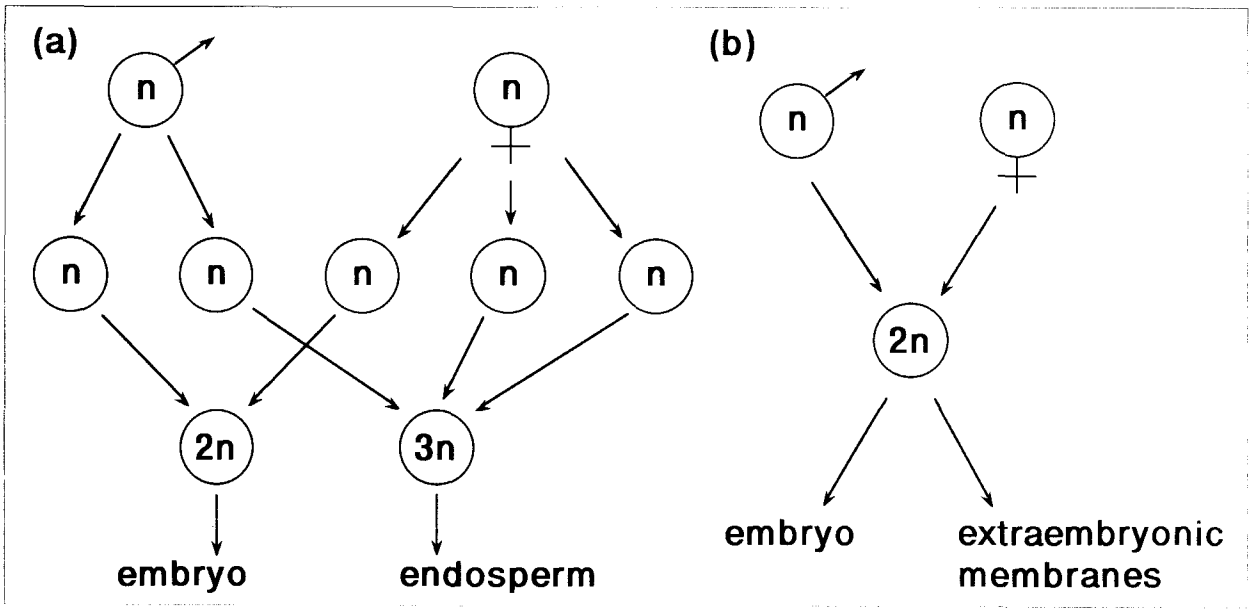

FIG 1

Genetic make-up of (a) flowering plant embryo and endosperm, and (b) mammalian embryo proper and extraembryonic membranes.

endosperm has a paternal excess (2m:2p) and undergoes greater mitotic activity than control endosperms (2m:1p, 4m:2p). When the diploid is the pollen parent, the endosperm has a maternal excess (4m:1p), mitotic activity is reduced and maternal tissue remains unconsumed within the kernel[9]. Similar reciprocal differences in interploidy crosses have been reported from a wide taxonomic range of angiosperms[10].

Analogous data come from the experimental manipulation of mouse development and clinical observations of humans. The extraembryonic membranes of gynogenetic and parthenogenetic mouse embryos (2m:0p) develop poorly, but early development of the embryo itself is more or less normal. Androgenetic mouse embryos (0m:2p), in contrast, undergo little embryonic development but have well-developed membranes. Both types of embryos abort[11]. Similarly, human triploid foetuses develop a large placenta if the extra genome is paternal, but have little placental tissue if the extra genome is maternal[12].

1m:1p	2m:2p
	3m:2p
2m:1p	4m:2p
	5m:2p
3m:1p	6m:2p
4m:1p	
5m:1p	
6m:1p	
7m:1p	

FIG 2
Genomic constitution of maize endosperm (after Lin[6]); the ratio (m:p) is the number of maternal and paternal genomes; underlining indicates normal development; dotted underlining indicates small kernels; all other combinations give abortive development.

Several chromosome deletions in humans and uniparental disomies in mice indicate that the expression of some genes in these regions depends on parental origin. The most striking example in humans is deletion of 15q11–13. Maternal loss of this segment is associated with Angelman syndrome, whereas paternal loss is associated with Prader–Willi syndrome[13]. In mice, disomy for the maternal chromosome 11 results in neonates that are smaller than normal, whereas neonates that are disomic for the paternal homologue are larger than normal. Offspring with maternal disomy for chromosome 2 are less active than normal mice, whereas offspring with the corresponding paternal disomy are more active[14].

Evolution of imprinting

Haig and Westoby have proposed an adaptive explanation to account for the similarities between imprinting phenomena in mammals and angiosperms[15]. Hall's proposal that imprinting has evolved in mammals to restrain the proliferative growth of the placenta is similar[12]. Haig and Westoby's argument is summarized below.

Consider the relationship between a pregnant mouse and her litter. The more resources an embryo acquires from its mother, the larger it is at birth, and the more likely it is to survive and reproduce. However, the greater the nutrient demand of the pregnancy, the greater the cost to the mother's potential future reproduction. This creates a conflict between the interests of the maternal and paternal genes within an embryo because the mother's future offspring will sometimes have a different father. A similar argument applies if there is mixed paternity within litters.

Some genes that are expressed in embryos will influence the amount of resources that are transferred from a mother to her current offspring at the expense of future offspring. A good example would be a gene for a placental growth factor. A new mutant allele that causes increased nutrient demands on the mother, but only when the allele is paternally derived, has a selective advantage over an established nonimprinted allele because the imprinted allele acquires extra nutrients for its own embryo at the expense of maternal half-sibs. Full genetic models have not been developed but the prediction is clear. If a locus has preferential paternal expression it will function to increase the nutrient demands on the mother; preferential maternal expression reduces those demands. This prediction is in broad agreement with the observations described earlier.

In summary, it is proposed that imprinting has evolved in mammals and flowering plants because their offspring are nourished directly from maternal tissues. As a consequence, genes that are expressed in an embryo or seed can influence the quantity of resources that the offspring receives from its mother. Significant effects of imprinting are not expected during embryonic development in oviparous taxa because the amount of yolk is usually determined before fertilization or immediately afterwards. Thus, genes expressed in embryos cannot influence the amount of resources they receive from their mother. This may explain why parthenogenesis has been observed in all major groups of vertebrates except mammals.

Alternative ideas have been put forward to account for the evolution of imprinting (discussed in Ref. 12). Perhaps the most persistent proposal has been that imprinting has evolved to prevent parthenogenesis. Parthenogenetic reproduction is often assumed to have short-term advantages for females but longer-term disadvantages for the species. If this is true, the proposal cannot explain how selection for the short-term benefit of parthenogenesis is overridden by selection for the long-term benefits of sexual reproduction. Other proposals suggest that imprinting allows flexible gene expression and sophisticated control of development. None of these hypotheses explains why, among vertebrates, mammals alone should require such mechanisms, nor the frequent association of imprinted genes with growth abnormalities. Neither do they suggest a reason for the strong correlation of paternal overexpression with increased growth, and maternal overexpression with reduced growth. Our hypothesis predicts precisely this pattern of expression.

Predictions for mammalian development

The theory outlined above proposes that imprinting has evolved because of the conflicting interests of maternal and paternal genes within offspring. However, genetic conflict over the amount of resources an offspring obtains from its mother is perfectly compatible with the two genomes cooperating to produce a viable offspring, because both sets of genes have a

common interest in the offspring surviving to reproduce. We believe that a mammalian mother probably maintains overall control of the amount of energy invested in individual offspring and that imprinting operates at the margins of this control, with paternal genes programmed to obtain as much nourishment as possible for the embryo and maternal genes programmed to counter this effect.

At fertilization, the egg and sperm are predicted to carry different genetic programmes that were established in the parental germ lines. However, these programmes are unlikely to go uncontested in the early embryo because either genome could benefit from modifying the other's programme. The maternal genome would have a strong advantage in reprogramming because the mother contributes virtually all of the egg's cytoplasm, but transcription factors or the like could possibly be introduced with the sperm. The evidence we have reviewed shows that reprogramming, if it occurs, cannot be completely effective.

There is evidence for genetic modification both before and after fertilization. Transgenes are differentially methylated during spermatogenesis and oogenesis in mice, and methylation is further modified in the egg cytoplasm after fertilization[2]. Shortly after sperm penetration, the sperm head undergoes rapid decondensation while the egg chromosomes are still highly condensed[16]. Paternal genes could be modified by maternally encoded enzymes at this stage. On the other hand, proto-oncogenes that are expressed in haploid spermatids have been suggested to have a regulatory function in the egg after fertilization[17]. Two further observations suggest that differential imprinting could occur during the early cleavage stages: there are high levels of methylase in the egg cytoplasm (W. Reik, pers. commun.), and paternal chromosomes are localized to one region of the nucleus during early interphases[18].

We further predict that most imprinted genes will affect how much an offspring receives from its mother, at the expense of its sibs. Thus, imprinting is expected at loci that influence placental growth, suckling, neonatal behaviour, appetite, nutrient metabolism and postnatal growth rate. We tentatively suggest that many of the behavioural abnormalities associated with duplications or deletions of imprinted loci are based on the dysfunction of adaptations related to suckling, teat acquisition, attracting maternal attention, or interactions among sibs. We believe it is worth considering the possibility that imprinting influences appetite control and hypothalamic function.

Interesting clues are provided by the relative contribution of parthenogenetic cells to different tissues of mouse chimaeras consisting of normal and parthenogenetic cells. Parthenogenetic cells make a disproportionately small contribution to the extraembryonic membranes, skeletal muscle (including the tongue), liver and pancreas[19,20], and birth weights of chimaeric mice are negatively correlated with the proportion of parthenogenetic cells[20]. Such observations contrast with the clinical manifestations of Beckwith–Wiedemann syndrome in humans. This syndrome is associated with large placentas, heavy birth weights, excessive growth through adolescence, large tongues, large livers and hyperplasia of the islets of Langerhans[21]. Other chromosomal syndromes are associated with abnormalities of suckling. A 'typical' case of Angelman syndrome (maternal deletion of 15q11) has 'uncoordinated tongue movements, suck, and swallow, as well as frequent vomiting', whereas a 'typical' case of Prader–Willi syndrome (paternal deletion of 15q11) has 'poor suck leading to severe failure to thrive'[13].

X-chromosome inactivation

Female mammals have a maternally derived X chromosome (X^m) and a paternally derived X chromosome (X^p) in each cell. One of these undergoes a process of inactivation early in development, the function of which is generally assumed to be the equalization of X-linked gene dosage between female cells and male cells (which have only X^m). Two patterns of X inactivation have been described. (1) Paternal X inactivation; X^p is inactivated in the trophectoderm and primitive endoderm of mice (and the cytotrophoblast of humans[22]), and in the somatic cells of female marsupials. (2) Random X inactivation; X^m and X^p are inactivated at random in different cells of the somatic tissues of female eutherians[23,24]. Can genomic imprinting help to explain the distribution of the two types of inactivation?

Random X inactivation only equalizes gene dosage between the sexes if X^m and X^p have the same expression. Otherwise, dosage compensation requires inactivation of X^p. The extraembryonic membranes of eutherians are subject to imprinting, and these tissues do in fact show paternal X inactivation. Some degree of imprinting would also be expected in somatic tissues, yet X inactivation is random in the soma of female eutherians. Two factors possibly lessen the force of this counterargument. First, most somatic gene expression probably does not involve a tradeoff between an individual's fitness and that of its maternal half-sibs, whereas most placental gene expression would have such an effect. Second, growth differences established before birth could have a major influence on subsequent growth rates, such that gene expression before birth could be the major determinant of the overall nutrient demand on the mother, even though a greater total transfer of resources occurs after birth. Given the absence of significant imprinting of X-linked genes, random inactivation has the advantage that deleterious alleles are expressed in only a subset of cells[5]. This would benefit genes on both X chromosomes. X^p is preferentially inactivated in the somatic cells of female marsupials[24], suggesting that imprinting is more important during postnatal development in marsupials than in eutherians. Significantly, the overwhelming majority of nutrient transfer in marsupials occurs after birth, during lactation.

These arguments suggest that paternal X inactivation is 'imposed' on X^p, but random X inactivation may be 'self-enforced'. In the trophectoderm of triploid mice, X^p is always inactive and X^m always active, irrespective of the number of each present (for $X^mX^mX^p$, X^mX^mY, $X^mX^pX^p$ and X^mX^pY). Thus, inactivation is based on parental origin and does not involve 'counting' of the number of active X chromosomes. On the other hand, only one X is active in the embryonic ectoderm of triploid mice, and this can be either X^m or X^p (Ref. 25).

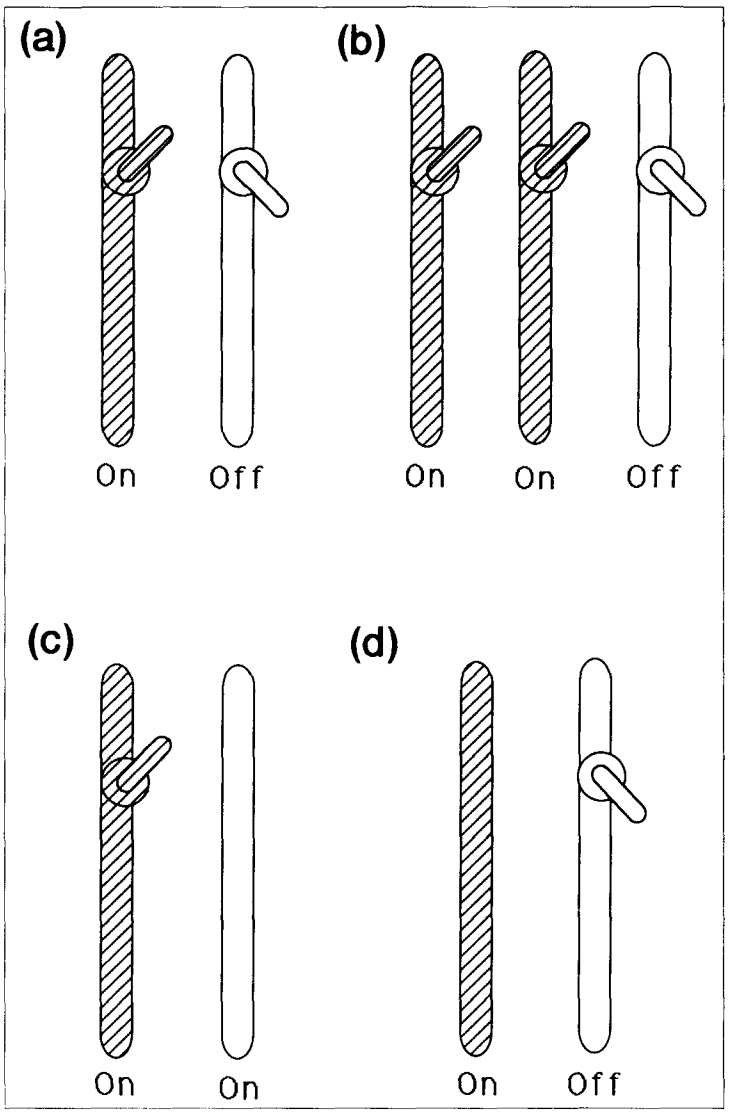

FIG 3

Model of Beckwith–Wiedemann syndrome (BWS). (a) In normal development, the paternal copy of IGF-II (hatched) is switched on and the maternal copy (unhatched) is switched off. (b) In sporadic cases of BWS, the paternal locus is duplicated to give two active copies of the locus. In BWS pedigrees, there is a mutant allele that cannot be switched off when maternally derived. Therefore (c) heterozygotes have two active copies and BWS when this allele is maternally derived, but (d) one active copy when the allele is paternally derived.

It may be significant that dosage compensation has not been reported in oviparous birds and reptiles. These are groups in which imprinting is expected to be relatively unimportant. Similarly, increased transcription of X^m in males, as occurs in *Drosophila*, would not be an effective mechanism of dosage compensation if X^p were imprinted. Our hypothesis does not require that imprinted genes occur on the X chromosome; rather, we predict that imprinting at X-linked loci would rapidly evolve, with possible adverse effects, if X inactivation were random in those tissues that now have paternal X inactivation.

A case study

Sporadic cases of Beckwith–Wiedemann syndrome (BWS) are associated with paternal duplication of 11p15 (Refs 3, 26). Familial BWS also maps to 11p15 but is associated with maternal transmission[27]. Loci that map to this region include the genes for insulin and insulin-like growth factor-II (IGF-II) and *HRAS1* (Ref. 28). The IGF-II peptide is believed to stimulate the growth of undifferentiated cells[29,30], and inactivation of the paternal copy of the gene causes growth retardation in mice[31]. Thus, overproduction of IGF-II could plausibly explain the foetal overgrowth that is characteristic of BWS. If expression at the locus were predominantly paternal, maternal duplications would have little effect on phenotype but paternal duplications would cause foetal overgrowth. Familial cases could be explained by inheritance of an allele that is not switched off when maternally inherited (Fig. 3).

IGF-II interacts with two structurally unrelated receptors. Most of its growth-promoting effects appear to be mediated through the IGF-I receptor, which also binds IGF-I and insulin. However, IGF-II has greater affinity for the IGF-II receptor, which is the same molecule as the cation-independent mannose 6-phosphate receptor (CI-MPR)[30,32]. As the CI-MPR, this protein transports enzymes, and macromolecules to be degraded, into lysosomes[33]. In mouse embryos, transcripts of CI-MPR are only produced from the maternal copy of the locus[34]. We propose that an important function of the CI-MPR is to facilitate the degradation of paternally produced IGF-II. Consistent with this hypothesis, antibodies that block the receptor's binding site for IGF-II do not inhibit biological responses to IGF-II in cultured myoblasts, but do inhibit IGF-II degradation[35]. The CI-MPR has recently been shown[36] to regulate the activity of a G protein in response to IGF-II, suggesting that the CI-MPR has acquired some signalling functions in addition to its role in degradation.

The paradoxical binding of IGF-II to the CI-MPR makes sense if this is seen as an evolved maternal response to paternal production of IGF-II. In this scenario, an initially fortuitous affinity of IGF-II for the CI-MPR would have been exploited by the maternal genome to compete for IGF-II with the IGF-I receptor. Significantly, the CI-MPR does not bind IGF-II in chickens or in *Xenopus*[37]. This is consistent with our hypothesis because IGF-II is not expected to be subject to imprinting in oviparous vertebrates.

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References

1 Solter, D. (1988) *Annu. Rev. Genet.* 22, 127–146
2 Surani, M.A., Reik, W. and Allen, N.D. (1988) *Trends Genet.* 4, 59–62
3 Reik, W. (1989) *Trends Genet.* 5, 331–336
4 White, M.J.D. (1973) *Animal Cytology and Evolution* (3rd edn), Cambridge University Press
5 Chandra, H.S. and Brown, S.W. (1975) *Nature* 253, 165–168
6 Lin, B-Y. (1984) *Genetics* 107, 103–115
7 Lin, B-Y. (1982) *Genetics* 100, 475–486
8 Birchler, J.A. and Hart, J.R. (1987) *Genetics* 117, 309–317
9 Cooper, D.C. (1951) *Am. J. Bot.* 38, 702–708
10 Wangenheim, K-H.v. (1962) *Z. Vererbungslehre* 93, 319–334
11 Barton, S.C., Surani, M.A.H. and Norris, M.L. (1984) *Nature* 311, 374–376
12 Hall, J.G. (1990) *Am. J. Hum. Genet.* 46, 857–873
13 Magenis, R. E. *et al.* (1990) *Am. J. Med. Genet.* 35, 333–349
14 Cattanach, B.M. and Kirk, M. (1985) *Nature* 315, 496–498
15 Haig, D. and Westoby, M. (1989) *Am. Nat.* 134, 147–155
16 Wright, S.J. and Longo, F.J. (1988) *J. Exp. Zool.* 247, 155–165
17 Propst, F., Rosenberg, M.P. and Vande Woude, G.F. (1988) *Trends Genet.* 4, 183–187
18 Odartchenko, N. and Keneklis, T. (1973) *Nature* 241, 528–529
19 Fundele, R.H. *et al.* (1990) *Development* 108, 203–211
20 Paldi, A. *et al.* (1989) *Development* 105, 115–118
21 Pettenati, M.J. *et al.* (1986) *Hum. Genet.* 74, 143–154
22 Harrison, K.B. (1989) *Cytogenet. Cell Genet.* 52, 37–41
23 Grant, S.G. and Chapman, V.M. (1988) *Annu. Rev. Genet.* 22, 199–233
24 Cooper, D.W., Johnston, P.G., VandeBerg, J.L. and Robinson, E.S. (1990) *Aust. J. Zool.* 37, 411–417
25 Endo, S., Takagi, N. and Sasaki, M. (1982) *Dev. Genet.* 3, 165–176
26 Brown, K.W., Williams, J.C., Maitland, N.J. and Mott, M.G. (1990) *Am. J. Hum. Genet.* 46, 1000–1001
27 Koufos, A. *et al.* (1989) *Am. J. Hum. Genet.* 44, 711–719
28 Junien, C. and McBride, O.W. (1989) *Cytogenet. Cell Genet.* 51, 226–258
29 Heyner, S., Smith, R.M. and Schultz, G.A. (1989) *BioEssays* 11, 171–176
30 Sara, V.R. and Hall, K. (1990) *Physiol. Rev.* 70, 591–614
31 DeChiara, T.M., Efstratiadis, A. and Robertson, E.J. (1990) *Nature* 345, 78–80
32 Morgan, D.O. *et al.* (1987) *Nature* 329, 301–307
33 Kornfeld, S. and Mellman, I. (1989) *Annu. Rev. Cell Biol.* 5, 483–525
34 Barlow, D.P. *et al. Nature* (in press)
35 Kiess *et al.* (1987) *J. Biol. Chem.* 262, 12745–12751
36 Murayama, Y. *et al.* (1990) *J. Biol. Chem.* 265, 17456–17462
37 Clairmont, K.B. and Czech, M.P. (1989) *J. Biol. Chem.* 264, 16390–16392

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Nuclear targets for transcription regulation by oncogenes

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Recent discoveries have highlighted the importance of transcription in cellular transformation. Transcription factors have a crucial role as nuclear targets that convert mitogenic signals from oncogenes into changes in gene expression.

Oncogene expression is a decisive event in cell transformation, resulting in altered transcription of certain genes. An emerging unifying theme is that different proto-oncogene products function as components of a network that transduces signals from the exterior of the cell to transcription factors in the nucleus. These proto-oncoproteins have various biochemical functions, for example as growth factors, their receptors and kinases. They may be located outside the cell, on the plasma membrane, or in the cytoplasm (see Fig. 1 and Refs 1, 2). There has been considerable recent progress in understanding the interactions among the proteins outside the nucleus[3]; the purpose of this review is to describe the nuclear targets for this network. Since the initial discovery that Fos and Jun are transcription factors, a number of other oncoproteins have also been shown to be transcription factors. We will discuss the evidence that these and other factors lie on the signalling network, and how they may interact to generate the transcriptional response to growth stimuli.

Oncoproteins that interact with oncogene-responsive elements

Regulation of transcription from promoters is mediated by a number of short sequence elements that specifically bind transcription factors. In promoters whose activity is altered by oncogene expression, some of these elements confer the oncogene responsiveness. Interestingly, a growing number of the proteins that bind to these responsive elements are turning out to be oncogene products. The first of these were Jun and Fos.

Jun and Fos

AP-1 is a dimeric transcription factor composed of proteins belonging to two different families of proto-oncogene products: Jun and Fos (Table 1)[4,5]. Dimer formation is necessary for DNA binding, and results from interaction between the leucine repeats of Fos and Jun. Leucine repeats are α-helical domains, formed by four or five leucines separated by six amino acids, which control the specificity of dimerization. Fos cannot dimerize with itself and thus does not bind independently to DNA; only Jun–Jun and Jun–Fos dimers can form. Heterodimers are more stable than homodimers, thus accounting for their much greater DNA-binding affinity. Members of the Jun family can dimerize with each other and with every member of the Fos family, giving 18 different combinations.