the unhindered stimulus to prolactin secretion may cause the development of a tumorous pituitary in rats. There is no evidence that prolonged exposure to estrogen is responsible for development of prolactinomas in humans (*16*). However, there is some evidence that dopaminergic activity may be reduced in prolactinemic patients (*17*) and that catecholamines are decreased in the brains of elderly individuals (*18*). Hence, damage to TD neurons may be associated with the development of human prolactinomas (*1*).

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Chromosomal Localization of the Human Homolog (*c-sis*) of the Simian Sarcoma Virus *onc* Gene

Abstract. *Nonrandom chromosome rearrangements of chromosome 22 have been identified in different human malignancies. As a result of Southern blot hybridization of a* c-sis *probe to DNA's from mouse-human somatic cell hybrids, the human homolog* (c-sis) *of the transforming gene of simian sarcoma virus was assigned to chromosome 22. Hybrids between thymidine kinase–deficient mouse cells and human fibroblasts carrying a translocation of the region q11-qter of chromosome 22 to chromosome 17 were also analyzed. These studies demonstrate that the human* c-sis *gene is on region 22q11>qter.*

The transforming genes of oncogenic retrovirus are derived from a set of cellular sequences known as cellular *onc* genes (*1*). These sequences are present in the genome of vertebrate species; their high degree of evolutionary conservation suggests that they code for proteins that are essential for cellular metabolism or for tissue differentiation (or both) (*1*). In one well-studied case it has been shown that the product of the cellular *onc* gene is very closely related to that of the viral *onc* gene (*2*). Moreover, the *onc* gene product is present at abnormally high levels in infected transformed cells (*2*). Thus, as an alternative to direct transformation by a viral *onc* gene, abnormal activation of a cellular *onc* gene may be involved in neoplastic transformation. At least three models can be proposed for such a mechanism. First, high levels of expression of a cellular *onc* gene may be caused by the insertion nearby of a viral promoter (*3*) or by alteration of the physiologic promoter by a mutagenic agent such as a chemical carcinogen. Second, a cellular *onc* gene may be expressed at high levels as a consequence of gene amplification (*4*). Third, a cellular *onc* gene may be relocated in a transcriptionally active region of the genome (*5*) as a consequence of chromosomal rearrangements. Since nonrandom chromosome rearrangements have been identified in several human malignancies (*6*), we can test the latter hypothesis by determining the chromosomal location of various human homologs of viral *onc* genes in normal and neoplastic cells. We now report on the chromosomal localization of the human homolog (*c-sis*) of the transforming gene (*v-sis*) of simian sarcoma virus (*7*). Knowledge of the chromosomal location of the *c-sis onc* gene should make it possible to determine whether the human *c-sis onc* gene can be activated by chromosome translocation or rearrangement in human malignancies.

We have shown that sequences homologous to the transforming gene of simian sarcoma virus are present in the

Table 1. Presence of the human *c-sis* gene in hybrid clones.

Human chromosome	Number of hybrid clones that are*			
	+/+	+/−	−/+	−/−
1	1	9	2	19
2	0	10	3	18
3	3	7	3	18
4	0	10	4	17
5	0	10	2	19
6	5	5	3	18
7	1	9	7	14
8	3	7	2	19
9	1	9	4	17
10	1	9	1	20
11	3	7	4	17
12	2	8	10	11
13	2	8	2	19
14	8	2	10	11
15	3	5	4	19
16	2	8	1	20
17	3	7	6	15
18	3	7	2	19
19	1	9	6	15
20	3	7	2	19
21	2	8	2	19
22	10	0	0	21
x	4	6	2	19

*+/+, Clones that contain the *c-sis* gene and the numbered chromosome; +/−, clones that contain the *c-sis* gene but not the numbered chromosome; −/+, clones that contain the numbered chromosome and do not contain the *c-sis* gene; −/−, clones that contain neither the numbered chromosome nor the *c-sis* gene.

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genome of vertebrate species (*8*). The human *c-sis* gene was cloned and study of its genomic organization indicated that it is a single-copy gene spanning approximately 12 kilobases (kb) in which five *v-sis* homologous regions are interrupted by four introns (*9*). The protein product of *v-sis* has been immunologically identified (*10*) but the function and tissue distribution of its cellular counterpart is unknown. An increase of *c-sis* messenger RNA (mRNA) has been found in specific types of human neoplasias, notably sarcomas and glioblastomas (*11*). However, it is not yet clear whether the elevated *c-sis* expression correlated with neoplastic transformation or whether expression depends on a particular stage of cellular differentiation.

In order to determine the chromosome location of the human *c-sis* gene we have used the Southern blotting hybridization technique (*12*) to screen the DNA of different human-mouse somatic cell hybrid clones that have selectively lost different sets of human chromosomes. A *c-sis* DNA probe was constructed by subcloning a 1.7-kb Bam HI restriction fragment into the plasmid pBR322. This fragment comprises the 3′ exon of human *c-sis* homolog (*8, 9*). This probe hybridized to a 5.2-kb Bam HI fragment of mouse genomic DNA (Fig. 1). Thus the mouse and human fragments were distinguishable in Bam HI DNA digests (Fig. 1, lanes e and f), allowing us to screen different mouse-human cell hybrid DNA's (Fig. 1, lanes a to d). We have characterized 31 independent hybrid clones for the expression of isozyme markers assigned to each one of the different human chromosomes (*13*) and for the presence of the human *c-sis* 1.7-kb Bam HI fragment. The presence of the *c-sis* human homolog segregates concordantly with the presence of human chromosome 22.

In order to localize the *c-sis* gene on human chromosome 22 we have hybridized GM0119 human fibroblasts (from the Human Genetic Mutant Cell Repository, Camden, New Jersey) with LM-TK$^-$ mouse cells deficient in thymidine kinase. The GM0119 human fibroblasts carry a reciprocal translocation between chromosome 17 and chromosome 22, (17;22) (17qter >17p13:: 22q11 >22qter; 22pter >22q11:: 17p13>17pter). The hybrids were selected in HAT (hypoxanthine, aminopterin, thymidine) medium containing $10^{-5}M$ ouabain (*5*) and tested for the expression of mitochondrial aconitase, a marker for human chromosome 22 and for the presence of the chromosome involved in the reciprocal translocation. As shown in Table 2, the clones carrying the 17p+ chromosome contained the human *c-sis* homolog. Counterselection of three independent hybrid clones (clones 4, 5, and 6) in medium containing 5-bromodeoxyuridine (BrdU) (30 μg/ml) resulted in the concomitant loss of the 17p+ chromosome and the human *c-sis* gene. Therefore, we conclude that this human *onc* gene is located on region q11>qter of chromosome 22.

Table 2. Presence of the human *c-sis* homolog in LM-TK$^-$ × GM0119 hybrids.

Hybrid clones	Selection	$ACON_M$	Chromosomes*				*c-sis*
			17	17p+	22	22q$^-$	
Clone 1	HAT	−	+	−	−	−	−
Clone 2	HAT	−	+	−	−	−	−
Clone 3	HAT	−	+	−	−	−	−
Clone 4	HAT	+	+	+	−	−	+
Clone 4	BrdU	−	−	−	−	−	−
Clone 5	HAT	+	−	+	−	−	+
Clone 5	BrdU	−	−	−	−	−	−
Clone 6	HAT	+	−	+	−	−	+
Clone 6	BrdU	−	−	−	−	−	−
Clone 7	HAT	+	+	+	−	−	+

*17p+ = 17qter > 17p13:: 22q11 > 22qter; 22q$^-$ = 22pter > 22q11:: 17p13 > 17pter.

Increases in *c-sis* mRNA have been detected in various human tumor cell lines (*11*). The largest amount was shown in the human glioblastoma cell line A172 (*11*) and slightly less in different cell lines derived from osteosarcomas and fibrosarcomas (*11*). At present it is not known whether these tumor cell lines carry rearrangements of chromosome 22. Deletions of the long arm of chromosome 22 have been observed in human meningiomas (*14*). No information is available, however, on transcription and expression of the *c-sis* gene in these tumors.

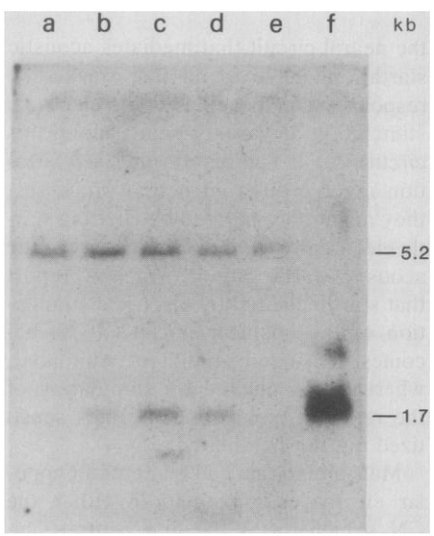

Fig. 1. Hybridization of human *c-sis* probe to hybrid and parental DNA. Lane e, mouse DNA; lane f, human DNA; lane a, negative hybrid clone. Lanes b, c, and d are positive hybrid clones.

Nonrandom rearrangements involving human chromosome 22 have also been described in human malignancies of the hematopoietic system (*6*). In chronic myelogenous leukemia a segment of the long arm of human chromosome 22 (q11>qter) is translocated to the long arm of human chromosome 9 (*6*). A similar rearrangement has been observed in cases of acute myelogenous leukemia and acute lymphocytic leukemia (*14*). In these cases the breakpoint has been localized at band 22q11 (*6, 14*). More recently, rearrangements of human chromosome 22 have been described in variant cases of Burkitt's lymphoma (*15*). In these variants, however, the segment 22q11>qter translocates to human chromosome 8 (*15*). Expression of the *c-sis* gene has not been generally observed in malignancies of the hematopoietic system (*11*). However, only a limited number of cases have been studied and there is no cytogenetic information available concerning these cases. Moreover, in one particular case, significant amounts of *c-sis* mRNA were detected in the T-lymphoma cell line, HUT102 (*11*), from which the human retrovirus HTLV was originally isolated (*16*). Thus, the *c-sis* gene can be activated in isolated cases of given malignancies. Information on the chromosomal location of human *onc* genes combined with data on their expression of these genes in tumor tissues should be helpful in determining whether there is a causal relationship between chromosomal changes and *onc* gene activation in human cancers.

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Habituation and Sensitization of Startle Reflexes Elicited Electrically from the Brainstem

Abstract. *Repetitive elicitation of startle-like responses by electrical stimulation of the cochlear nucleus led to sensitization followed by habituation. In contrast, repetitive elicitation of startle-like responses by electrical stimulation of the reticular formation led only to sensitization. Since these different locations represent different points along the acoustic startle circuit, the data suggest that sensitization may be related to the motor side of reflex arcs, whereas habituation may be related to the sensory side.*

Repetitive elicitation of reflex behavior is thought to involve both decremental (habituation) and incremental (sensitization) processes that interact to determine response strength (*1*). In invertebrates, the cellular mechanisms underlying habituation and sensitization are independent (*2*). In more complex vertebrate systems, the two processes are assumed to be mediated by different neuronal systems, although only a few direct demonstrations support this assumption (*3*). Habituation results from stimulus repetition; sensitization can be caused by either stimulus repetition or exposure to stimuli other than those used to elicit the reflex. The acoustic startle reflex in the rat shows habituation to a repeated acoustic stimulus but sensitization to high levels of background white noise or a repeated visual stimulus (*4*). Recently we have attempted to delineate the neural circuit that mediates acoustic startle and have found that startle-like responses can be elicited by electrical stimulation at various points along this circuit (*5*). If habituation and sensitization involve different neural processes, they might be separated by eliciting startle electrically from different parts of the acoustic startle circuit. We now report that startle elicited by electrical stimulation of the cochlear nucleus (CN) becomes sensitized and then habituated, whereas that elicited by stimulation of the reticulo-spinal tract becomes sensitized but not habituated.

Male albino rats had bilateral monopolar electrodes implanted in either the CN, which forms the first central synapse in the acoustic startle circuit, or in the nucleus reticularis pontis caudalis (RPC), whose cell bodies form the reticulo-spinal tract that mediates the motor side of startle (*6*). One week later, the rats were placed in cages in which startle reactions were recorded (*7*), and 1 minute later were given single stimuli (1 msec, 25 to 100 μA to each electrode) bilaterally in either the CN ($N = 8$) or the RPC ($N = 8$) every 30 seconds (*8*). A total of 60 stimuli were applied over the 30-minute test session. Throughout testing, a constant level of 80-dB background noise was maintained. In addition, control rats ($N = 8$) were tested for acoustic startle elicited by 60 50-msec, 110-dB tones spaced 30 seconds apart.

Figure 1A shows that startle elicited acoustically or electrically through the CN showed an initial increase in amplitude followed by a gradual decrease toward the end of the session [for acoustic stimuli $F(14, 98) = 2.68$, $P < .01$; for CN stimulation $F(14, 98) = 2.71$, $P < .01$]. In contrast, startle elicited through the RPC increased across the session and did not subsequently decline [$F(14, 98) = 4.24$, $P < .001$].

Under these conditions, startle behaved differently when electrically elicited through the CN or the RPC. The results with acoustic and CN stimulation are consistent with prior reports (*4*), in which it was theorized that repetitive presentation of the eliciting stimulus produced habituation, whereas concomitant exposure to background noise produced sensitization. The net decrease in startle amplitude across the session was thought to result because the decremental effects of habituation overcame the incremental effects of sensitization. The progressive increase in startle amplitude with repetitive RPC stimulation in our study could have resulted from (i) repetitive stimulation of the RPC itself producing sensitization but no habituation, leading to a net increase in startle across the session, and (ii) exposure to background noise producing sensitization which led to a progressive increase across the session, since RPC stimulation did not produce habituation.

Previous experiments (*4*) have shown that the amplitude of the startle reflex elicited by a single tone increased as the duration of prior exposure to background noise was increased. This facilitatory effect was greatest after 30 minutes of prior exposure to background noise. When startle-eliciting tones were delivered repetitively following a 30-minute exposure to background noise, startle showed a pronounced and relatively smooth decline in amplitude within the test session. That is, once sensitization to background noise had reached a maximum (after 30 minutes), a relatively pure

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